Isolation of Fungal phytopathogen with herbicidal activity targeting the invasive weed *Alternanthera philoxeroides*

Project report submitted to Sister Nivedita University in partial fulfilment of the requirement for the degree of

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Submitted By

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The entire dissertation work has been done under my supervision and this work has not been submitted elsewhere for a degree.

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CHAPTER 01: ABSTRACT

The uncontrolled expansion of aquatic weeds, particularly Alligator weed (*Alternanthera philoxeroides*), has become a major ecological concern, contributing to a sharp decline in biodiversity across numerous freshwater ecosystems around the world. These invasive, semi-aquatic plants are highly competitive and often dominate over native species by rapidly colonizing water bodies, disrupting natural ecological balances. One of the main responses to invasive weeds has been the widespread application of chemical herbicides. However, this method has led to unintended and harmful consequences. Over time, continuous herbicide use has resulted in the development of resistant weed species, making them even harder to control. Biological control, especially using plant-pathogenic fungi, presents a promising and environmentally responsible solution. Phytopathogenic fungi are microorganisms that infect plants and cause various diseases, including leaf spots, blights, wilting, and tissue decay. Although they are often considered threats to agriculture, their ability to selectively target specific host plants makes them ideal candidates for controlling invasive weeds without harming native species or the broader environment. This research aimed to explore the potential of phytopathogenic fungi as biological herbicides (mycoherbicides) to manage alligator weed infestations. Fieldwork was carried out in multiple aquatic sites across Kolkata, West Bengal, to collect infected plant samples showing symptoms of fungal attack. These samples were then brought to the laboratory, where fungal strains were isolated and cultured. Each isolate was tested for its phytotoxic effects using crude fungal metabolites to assess its potential as a natural herbicide. Out of the 15 fungal isolates obtained during the study, several showed promising herbicidal activity, as evidenced by clear symptoms of damage to alligator weed, including chlorosis, necrosis, and tissue breakdown. The most effective isolates—those with the highest disease severity—were selected for further morphological and microscopic analysis to determine their species identity. Among these, a particularly potent strain was successfully identified as belonging to the *Alternaria* genus.

CHAPTER 02: KEYWORDS & ABBREVIATIONS

2.1. Keywords

Aquatic weeds, alligator weed, biocontrol, bioherbicide

2.2. Abbreviations

Cm – centimeter, ml – milliliter,

CHAPTER 03: INTRODUCTION

Alligator weed (*Alternanthera philoxeroides*), a fast-growing aquatic perennial native to South America, is an invasive species causing ecological and agricultural damage in regions like West Bengal, India (Dutta and Basak et al., 2024). While the fungus has been considered a potential biocontrol agent in countries such as Australia, Brazil, and China, further long-term research is essential to develop it into a viable bioherbicide for managing alligator weed. Alligator weed is posing significant risks to Queensland's waterways, wetlands, and irrigated agriculture (*Alternanthera philoxeroides* (Mart.) Griseb.). It thrives in both aquatic and terrestrial environments, forming dense mats that impede water flow, degrade water quality, and outcompete native vegetation. These can be dislodged by water movement and these floating mats can restrict water flow in creeks, channels and drains; impede recreational water sports and boating access and damage pumping and irrigation equipment and other

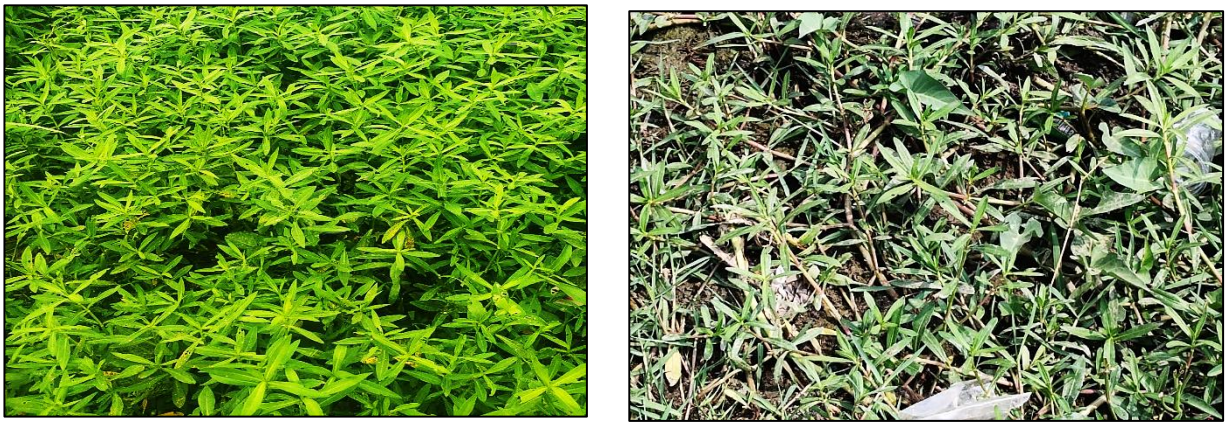


Fig.3.1. Alligator weed (*Alternanthera philoxeroides*) infested areas observed during field surveys.

structural features. It is known for its invasive nature and ability to spread quickly across water bodies, making it a major concern in many parts of the world (Holm, L.G., Plucknett, D.L., Pancho, J.V., & Herberger, J.P. (1977).). This plant thrives in both aquatic and semi-aquatic environments, often forming dense mats that can completely cover lakes, rivers, and wetlands. These dense mats block sunlight, reduce oxygen levels in the water, and hinder the movement of boats, fishing, and other recreational activities (Julien, M.H., & Stanley, J.N. (1999). Alligator weed has several defining characteristics: its broad, oval-shaped leaves, small white or pink flowers, and its ability to grow both in water and on land (Ghosh, S., & Biswas, P.K. (2002).). The stems are hollow when mature and can be single or branched. Stems can lie flat or upright. The flat stems can be light green, yellow or brown to red. Upright stems are dark green, can be up to 80 cm or longer. Stems have pairs of leaves at each node. It can propagate through seeds, fragments, and stolon's, which makes it especially difficult to control once established (Swarbrick, J.T. (1997). In many areas, alligator weed has been classified as a noxious weed due to its negative impact on biodiversity, water quality, and local economies. Alligator

weed can host a variety of pests and diseases in addition to having an adverse effect on the environment. Researchers have looked into the possibility of employing biological control techniques to control its spread because of its potential to harbor phytopathogens that can harm other plant species (Wheeler, G.S., & Center, T.D. (2001). Alligator weed was once brought to several nations as an ornamental plant or to prevent erosion, but it has since spread to many areas, including portions of Asia, Australia, and the United States, where it has become an invasive species. Efforts to manage and restrict its spread include chemical treatments, mechanical removal, and the use of biological control agents, but the plant's tenacity and capacity to regenerate from small fragments make it a persistent challenge. In different studies, a wide range of organisms including nematodes, insects, bacteria, and fungi are used as biocontrol agents for overcoming several of the aforementioned inadequacies. Biological control has been investigated, and four host-specific insect species from South America have been released in several part s of the world against this weed (Julien and Griffiths et al., 1998). Of these, the flea beetle *Agasicles hygrophila* and the moth Arcola malloi were introduced into the USA and are exerting good control of floating mats of the weed, except in the extreme tropical and temperate areas (Julien and Griffiths et al 1998; Stewart et al., 2000). Unlike its effectiveness in controlling floating mats, these insects have not controlled upland stands effectively. Hence, additional biocontrol agents are needed (Pomella, Robert & Raghavan et al., 2007). Scientific activity in mycoherbicide studies has increased significantly and application of registered or unregistered mycoherbicides has also expanded worldwide (Hamid Cheraghian Radi., et al. April 2020). Hence, there is a pressing need for environmentally friendly, cost-effective, and targeted biological solutions to manage invasive weed species. Certain plants, microorganisms, and fungi produce allelopathic compounds that can suppress weed growth. Among these, fungal pathogens and their metabolic byproducts have shown particular promise as biocontrol agents against water hyacinth and alligator weed.

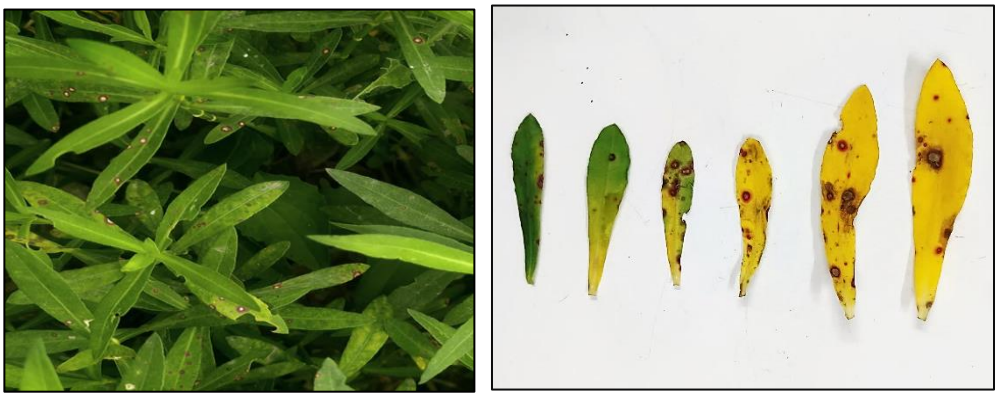


Fig.3.2. Alligator weed with disease symptoms observed and collected during the field survey.

CHAPTER 04: REVIEW OF LITERATURE

Invasive species like Alligator weed (*Alternanthera philoxeroides*) have posed significant threats to agricultural productivity and biodiversity (Holm, L.G., Plucknett, D.L., Pancho, J.V., & Herberger, J.P. (1977). Traditional control methods, including mechanical removal and chemical herbicides, often fall short due to cost, resistance development, and environmental hazards (Julien, M.H., & Stanley, J.N. (1999). As a result, there is growing interest in biological control agents, especially phytopathogenic fungi, for sustainable weed management. Several studies have documented the potential of fungi such as *Fusarium* spp., *Cercospora rodmanii*, *Colletotrichum gloeosporioides*, and *Myrothecium verrucaria* as bioherbicides. *Cercospora rodmanii* was one of the earliest fungi reported to cause significant damage to alligator weed in aquatic environments. (Templeton et al. (1979) demonstrated its effectiveness, but later studies revealed limited field success due to environmental sensitivity and formulation issues. The rapid spread of alligator weed (*Alternanthera philoxeroides*), a perennial aquatic and terrestrial invasive species, has prompted serious concerns globally, especially in wetlands, irrigation canals, and agricultural lands Conventional control methods—mechanical, chemical, and cultural—often fail to provide long-term, sustainable control. Consequently, biological control, particularly using phytopathogenic fungi, has emerged as a promising eco-friendly alternative. Early efforts in fungal biocontrol focused on identifying native or co-evolved pathogens that could suppress the weed's growth. *Cercospora rodmanii*, first isolated in the United States, was among the most notable fungi studied for this purpose. (Templeton et al. (1979) demonstrated that this pathogen could cause leaf spot and dieback in alligator weed under controlled conditions. However, its dependency on high humidity and temperature made large-scale field application inconsistent. In recent decades, attention shifted to fungi capable of producing active secondary metabolites with phytotoxic effects These metabolites, unlike whole fungal inoculants, offer advantages such as stability, specificity, and ease of formulation. One notable example is *Myrothecium verrucaria*, which produces a complex mixture of trichothecenes and macrolide-type toxins. (Research by Hoagland (2001) and (Boyette et al. (2002) confirmed that crude extracts of this fungus, even in the absence of viable spores, effectively killed alligator weed within days of application. The phytotoxic activity was attributed to the inhibition of ribosomal function, leading to cellular death. Recent studies also highlight the importance of screening fungal isolates for their host specificity to avoid non-target effects. research by (Yang et al. (2016) emphasized molecular identification of potent strains and chemical profiling of their metabolites using LC-MS and NMR techniques. Furthermore, encapsulation strategies such as alginate beads, nano emulsions, and UV-protective coatings have been developed to enhance the shelf life and field performance of fungal-based formulations. In conclusion, the exploration of phytopathogenic fungi and

their secondary metabolites presents a compelling approach for managing invasive species like alligator weed. Continued research is essential to bridge the gap between laboratory efficacy and field performance, as well as to navigate regulatory hurdles related to biosafety and environmental impact.

1. Invasiveness of *Alternanthera philoxeroides*

Alternanthera philoxeroides, commonly known as alligator weed, is a highly invasive plant species that poses significant ecological and economic challenges. Originally native to South America, it has spread globally, invading aquatic and terrestrial habitats (Julien et al., 1995). The plant's invasiveness is attributed to its rapid vegetative reproduction and tolerance to a wide range of environmental conditions. Dense infestations disrupt aquatic ecosystems by outcompeting native vegetation and reducing biodiversity. Furthermore, its presence in agricultural areas impacts crop productivity and water management systems (Ghosheh et al., 2020).

2. Conventional Management

Traditional management methods for alligator weed include mechanical, manual, and chemical approaches. Mechanical control involves the use of machinery to physically remove the plant, but this method is labor-intensive and often results in regrowth due to the resilience of its root systems. Manual removal is more targeted but impractical for large-scale infestations. Chemical control using herbicides like glyphosate and 2,4-D is effective for short-term management; however, it poses risks such as environmental pollution and non-target damage (Wang et al., 2016). These limitations necessitate the development of integrated management strategies.

3. Biological Control

Biological control has proven to be a sustainable solution for managing Alternanthera philoxeroides. The introduction of natural enemies, such as the alligator weed flea beetle (*Agasicles hygrophila*), has been effective in reducing weed biomass in several regions, particularly in tropical and subtropical climates (Coulson, 1977). Other biocontrol agents, including the thrip *Amynothrips_andersoni*, have also demonstrated success. Despite its potential, the effectiveness of biological control can be influenced by environmental factors and the adaptability of the agents (Sharma et al., 2021).

4. Phytopathogenic Fungi as Herbicide

Phytopathogenic fungi offer an innovative and environmentally friendly approach to controlling alligator weed. Species such as *Alternaria alternantherae* have shown high specificity and efficacy in attacking the plant. These fungi cause significant damage by infecting and degrading the weed's tissues,

leading to its decline (Li et al., 2019). The use of fungal bioherbicides is advantageous due to their minimal impact on non-target species and compatibility with integrated weed management systems. Further research is needed to optimize formulations and application methods for large-scale use.

5. International Approaches

Globally, various strategies have been implemented to manage *Alternanthera philoxeroides*. Australia and the United States have successfully utilized biological control programs, including the introduction of *Agasicles hygrophila*. Collaborative efforts between countries have facilitated knowledge exchange and improved management practices (Julien & Broadbent, 1980). These international initiatives highlight the importance of coordinated approaches in addressing invasive species.

6. National Approaches

In India, integrated weed management programs targeting alligator weed are gaining momentum. These programs combine mechanical removal, herbicide application, and biological control methods to address infestations effectively. Government initiatives, supported by research institutions, aim to develop sustainable and cost-effective solutions, including the use of indigenous biological agents (Patel et al., 2019). Community involvement and awareness campaigns further strengthen national efforts to combat this invasive species.

CHAPTER 05: OBJECTIVES

1. Isolation of Phytopathogenic Fungi from Infected Alligator Weed Leaves

2. Assessment of Toxicity on Healthy Alligator Weed Plants

3. Identification of Fungi with Herbicidal Properties Against Alligator Weed

4. Mass Culture of Isolated Fungi for Secondary Metabolite Production

CHAPTER 06: MATERIALS AND METHODS

6.1 Isolation of phytopathogenic fungus from infected alligator weed leaves

To obtain pure cultures of fungi responsible for diseases in *Alternanthera philoxeroides* (alligator weed), study was made to encompass sample collection, media preparation, leaf sterilization, and fungal inoculation was employed.

6.1.1 Sample Collection

Between January and March 2025, field surveys were conducted in Kolkata and neighboring districts—North 24 Parganas, South 24 Parganas, Howrah, and Birbhum—to identify alligator weed that were infected with disease symptoms**.**

Survey Site	Latitude(°N)	Longitude (°E)	Laboratory Isolation Code
Dum Dum, North 24 Parganas	22.619762	88.426483	ALI 4, ALI 15
Dumurjola Stadium, Howrah	22.582101	88.306739	ALI 2, ALI 3, ALI 9
Joka, South 24 Parganas	22.448200	88.301096	ALI 8
Kudghat, Kolkata	22.473288	88.344472	ALI 11, ALI 12, ALI 14, ALI 16
Kopai, Birbhum	23.712552	87.712555	**ALI 5, ALI 6, ALI 7, ALI 10, ALI 13**
New Alipur, Kolkata	22.508738	88.323594	No Isolates Obtained
Ramrampur, South 24 Parganas	22.272699	88.265591	No Isolates Obtained
Satragachi Jheel, Howrah	22.583195	88.294239	No Isolates Obtained

Table 6.1. List of alligator weed-infested sites along with fungal isolates obtained from diseased leaves of alligator weed in districts of West Bengal, India from January 2025 to March 2025

Procedure

1. Diseased alligator weed leaves showing visible symptoms were periodically collected from infested sites.
2. Samples were wrapped in layers of dry paper bags to prevent moisture accumulation. (Dutta, Ray, and Ray 2015)
3. The wrapped specimens were placed in paper bags and transported to the laboratory (Souza et al. 2017) using ice packs to maintain a cool environment, minimizing microbial growth during transit.

6.1.2 Sterilization of Infected Leaves

To ensure that only internal fungi are isolated, surface sterilization of the infected leaves was performed.

Materials Required

- Distilled water
- 70% Ethanol
- Sterile Petri dishes
- Sterile scalpel
- Sterile tweezers

Procedure:

1. Infected leaves were washed under running tap water to remove debris from surface (Schaad, N.W., Jones, J.B., & Chun, W. (2001).
2. Leaves were immersed in 70% ethanol for 30 seconds to disinfect the surface (Singleton, P., & Sainsbury, D. (2001)
3. Leaves were rinsed 2–3 times with distilled water to remove ethanol residues (Agrios, G.N. (2005).
4. Then the leaves were treated with 0.5% NaOCl solution for 30 seconds for further sterilization.
5. It was again rinsed with distilled water to remove any remaining sterilant (Harman, G.E., & Kubicek, C.P. (1998)
6. Using sterile scalpel and tweezers, small sections (including necrotic or chlorotic lesions) was excised from the sterilized leaves for inoculation (Campbell, C.L., & Madden, L.V. (1990)

6.1.3 Preparation of potato dextrose agar media

To prepare the culture media, it was subjected to steam sterilization in an autoclave at 121°C and 15 psi for 15 minutes. This process effectively eliminates microbial contaminants by exposing the media to high-pressure saturated steam. After sterilization, the media was aseptically poured into sterile Petri dishes within a laminar airflow cabinet. The plates were left partially open to allow the media to cool and solidify under sterile conditions.

Materials Required

• Erlenmeyer flask

• Sterile Petri plates

• Distilled water

• Potato dextrose agar

• Sterile inoculation loop

• Ethanol 99%

• Spirit lamp

• Autoclave & Laminar air flow cabinet

• Non-absorbent Cotton & permanent marker pen

Composition

SERIAL NUMBER	NUMBER OF CONSTITUENTS	AMOUNT (gm/Lt)
1	**Potato Infusion**	**200**
2	**Dextrose**	**20**
3	**Agar**	**15**

Table 6.2: - Composition of Potato Dextrose Agar

Procedure

1. All glassware were thoroughly cleaned and dried using a hot air oven (Schaad, N.W., Jones, J.B., & Chun, W. (2001)
2. 1000 mL of PDA medium was prepared in an Erlenmeyer flask by dissolving the appropriate quantities of the components (Agrios, G.N. (2005).
3. Sterilization of the medium in an autoclave at 121°C and 15 psi for 15 minutes was ensured (Pelczar, M.J., Chan, E.C.S., & Krieg, N.R. (1993)
4. After sterilization, under aseptic conditions within a laminar airflow cabinet, the hot medium was poured into sterile Petri dishes (Bailey, K.L., & Lazarovits, G. (2003)
5. The medium was allowed to cool and solidify with the lids partially open to prevent condensation (Atlas, R.M., & Snyder, J.W. (2006).

6.1.4 Inoculation of diseased leaves

The leaf parts that were sterilized properly were cultured on PDA to isolate and identify fungal pathogens.

Materials Required:

- Sterile inoculation loop
- Spirit lamp
- Autoclave and laminar airflow cabinet
- Non-absorbent cotton
- Permanent marker

Procedure:

1. Under aseptic conditions within a laminar airflow cabinet, the inoculation loop was flame sterilized using a spirit lamp ((Patel et al., 2018).
2. The sterilized leaf was placed on the surface of solidified PDA in Petri dishes ((Chen & Kumar, 2020).
3. Petri dishes were properly sealed with parafilm and labelled appropriately using a permanent marker ((Ahmed et al., 2017).
4. The plates were kept at an appropriate temperature (usually around 25–28°C) for several days, monitoring for fungal growth ((Singh & Rao, 2021).

6.1.5 Subculturing to obtain Pure Fungal Cultures

Subculturing maintains fungal cultures in an active state and ensures their longevity.

Materials Required:

- Sterile inoculation loop
- Spirit lamp
- Autoclave and laminar airflow cabinet
- Non-absorbent cotton
- Permanent marker
- Sterile distilled water
- Potato Dextrose Agar (PDA) media

Procedure:

1. All glassware were thoroughly cleaned and dried using a hot air oven (Schaad, N.W., Jones, J.B., & Chun, W. (2001).)
2. PDA media was prepared by mixing the required components with distilled water in an Erlenmeyer flask and sterilize at 121°C and 15 psi for 15 minutes (Agrios, G.N. (2005)
3. The sterilized media was poured into sterile Petri dishes within a laminar airflow cabinet and allow it to solidify (Pelczar, M.J., Chan, E.C.S., & Krieg, N.R. (1993)
4. Each agar plate was poured with the corresponding strain number (Pelczar, M.J., Chan, E.C.S., & Krieg, N.R. (1993)
5. Inoculation loop was sterilized by flaming until red-hot, then cool it by dipping in 99% ethanol.
6. (Atlas, R.M., & Snyder, J.W. (2006)
7. Using the cooled loop, a small section of the fungal colony onto the fresh PDA plate was transferred.
8. Incubation of plates were done at 27℃ for 3–5 days.
9. The incubated plates were sealed with parafilm and stored at 4℃ for future use.

6.2 Assessment of toxicity test on healthy alligator weeds

The twig pathogenicity assay evaluates the virulence of fungal isolates on healthy alligator weed (*Alternanthera philoxeroides*).

Materials Required:

- Petri dishes
- Tissue paper
- Absorbent cotton
- Aluminum foil
- Tap water
- Sterile scalpel
- Forceps
- Sterile paintbrush
- Mortar and pestle
- Tween 20 solution

Procedure:

1. 1.Beakers were cleaned and wiped with 70% ethanol (Schaad, N.W., Jones, J.B., & Chun, W. (2001))
2. Tissue paper that was folded kept- at the bottom of each beaker and moisten with a few drops of tap water to maintain humidity (Agrios, G.N. (2005).
3. 9–10 cm long healthy twigs of alligator weed with 6–8 leaves were selected (Pelczar, M.J., Chan, E.C.S., & Krieg, N.R. (1993)
4. Surface sterilization of the twigs was done by immersing them in 70% ethanol (Harman, G.E., & Kubicek, C.P. (1998).
5. Wrapping the base of each twig with wet absorbent cotton and cover with aluminium foil shaped into a bulb was ensured to retain moisture (Bailey, K.L., & Lazarovits, G. (2003).
6. Scalpel was sterilized with 70% ethanol and excised 1 cm^2 sections from fungal colonies grown on PDA plates (Charudattan, R. (2001).
7. Mixing of the fungal sections with distilled water and Tween 20 solution using a mortar and pestle to create a uniform suspension (Freeman, S., & Rodriguez, R.J. (1993).
8. Using a sterile paintbrush, application of the fungal suspension evenly onto the leaves and stems of the alligator weed twigs (Atlas, R.M., & Snyder, J.W. (2006).
9. The inoculated twigs were kept into the moistened Petri dishes using forceps and cover with lids to maintain humidity (Campbell, C.L., & Madden, L.V. (1990)
10. The presence or absence of disease recorded. The severity of infection was evaluated using numerical rating from 0 to 3 where 0= foliage with no symptoms, 1= leaf yellowing 2= necrosis 3= senescence of leaf (Singleton, P., & Sainsbury, D. (2001).

6.3 Whole plant bioassay for alligator weed

A whole-plant bioassay serves as a comprehensive method for assessing the herbicidal potential of phytopathogenic fungi and their metabolites.

Materials Required

1. Healthy Alligator weed plants.
2. Isolated fungal colonies.
3. Mortar and Pestle.
4. Sterilized scalpel.
5. Sterilized forceps.
6. Sterilized paint brush.

7. Tween 20 solutions.
8. Distilled water.

Procedure

1. Beakers were cleaned and then wiped with ethanol (70%), then we folded clean tissue paper in a square and was placed inside the cleaned beaker. (Schaad, N.W., Jones, J.B., & Chun, W. (2001)
2. Alligator weed each measured 9-10 cm long with twigs containing 8-12 leaves were gently washed with tap water and was kept for acclimatizing in lab environment for 3-5 days (Schaad, N.W., Jones, J.B., & Chun, W. (2001)
3. After acclimatizing the healthy alligator plants ensuring the leaves are disease free were taken (Pelczar, M.J., Chan, E.C.S., & Krieg, N.R. (1993).
4. Few drops of tap water were poured on tissue to wet it a bit to ensure moist environment. Disease free leaves were surface sterilized with 70% ethanol (Harman, G.E., & Kubicek, C.P. (1998).
5. Then a scalpel was sterilized with 70% ethanol and one centimeter square of each colony was cut from agar plates (Bailey, K.L., & Lazarovits, G. (2003).
6. Distilled water as well as tween 20 solution was mixed to the fungal colonies and finely mixed with the help of mortar and pestle respectively for replica of alligator leaves(Charudattan, R. (2001).)
7. Then a sterilized brush properly cleaned and washed with 70% ethanol was taken and the fungal mixture was applied to the leaves and stem of alligator weed carefully and evenly (Freeman, S., & Rodriguez, R.J. (1993).
8. Then with the help of sterilized forceps the leaves were kept inside tap water filled Erlenmeyer's flask and it was covered with plastic wraps to hold the moisture content. (Atlas, R.M., & Snyder, J.W. (2006).
9. Observations were taken on an interval of 1 day for 7 days (3rd day, 5th day, 7th day) (Campbell, C.L., & Madden, L.V. (1990)
10. The presence or absence of disease were recorded. The severity of infection was evaluated using numerical rating from 0 to 3 where 0= foliage with no symptom, 1= leaf yellowing,2= necrosis, 3= senescence of leaf. (Singleton, P., & Sainsbury, D. (2001).

6.4 SECONDARY METABOLITES EXTRACTED FROM PHYTOPATHOGENIC FUNGUS WITH HERBICIDAL PROPERTIES AGAINST WATER HYACINTH

6.4.1 Mass Culturing of the infecting fungus

Materials Required

- Erlenmeyer flask (250 mL, 500 mL, or 1 L depending on scale)
- Cotton
- Sterile inoculation loop
- Bunsen burner
- Tissue paper
- Measuring cylinder
- Ethanol (70%)
- Potato dextrose Broth
- Distilled water
- Fungal culture (e.g., from PDA plate or slant)
- Aluminum foil
- Autoclave
- Orbital shaker (for aeration)

Composition of potato dextrose broth

Constituents	Amount (gm/lt)
Potato infusion	200 gm/lt
Dextrose	20 gm/lt

Table 6.2: - Composition of Potato Dextrose Broth

Procedure

A) Preparation of Potato Dextrose Broth

1. Cleaned and washed Erlenmeyer flask of 1litre was taken (Schaad, N.W., Jones, J.B., & Chun, W. (2001)
2. 1litre of distilled water was poured through measuring cylinder and pour in the flask (Cappuccino, J.G., & Sherman, N. (2014)
3. 24 g of Potato Dextrose Broth was added to the flask containing distilled water by weighing it on a weighing machine (Atlas, R.M. (2010).
4. Using the magnetic stirrer the mixture was stirred until dissolved uniformly (Pelczar, M.J., Chan, E.C.S., & Krieg, N.R. (1993))
5. Then, the flask was cotton plugged and was autoclaved at 121psi for 15 minutes (Harman, G.E., & Kubicek, C.P. (1998)

B) Inoculation of fungal strains on Potato Dextrose Broth

1. The different strains of alligator weed (ALI 2, ALI 3, ALI 4, ALI 5, ALI 6, ALI 7, ALI 9, ALI 10, ALI 11, ALI 12, ALI 13, ALI 14, ALI 15, ALI 16) respectively were inoculated in the above prepared PDB broth using sterile loop in front of flame (Agrios, G.N. (2005)
2. A loop full of culture in the sterile inoculation loop was taken and inoculated in the above labelled Erlenmeyer's flask containing PDB, and was mixed well and cotton plugging was done (Charudattan, R. (2001).
3. For each strain of alligator weed three replicas was made by performing inoculation each time (Charudattan, R. (2001).
4. The above inoculated strains were incubated at 27°C for 21 days (Singleton, P., & Sainsbury, D. (2001)

C) Incubation of the fungal samples

1. The procedure was conducted under aseptic conditions in a laminar airflow cabinet to minimize contamination risks (Freeman, S., & Rodriguez, R.J. (1993).
2. A fungal plug measuring approximately 1 cm in diameter was aseptically removed from a well-grown Potato Dextrose Agar (PDA) plate using a sterile instrument (Campbell, C.L., & Madden, L.V. (1990).
3. The plug was carefully transferred into a sterile Potato Dextrose Broth (PDB) medium to initiate fungal culture (Schippers, B., & Van Loon, L.C. (1987)
4. For experiments requiring uniform fungal growth, either a spore suspension or finely fragmented mycelia was used instead of a solid plug (Bailey, K.L., & Lazarovits, G. (2003)
5. These alternative inoculation methods enhanced consistency and reproducibility across replicates Oven-dried or freeze-dried (lyophilized) for long-term preservation and use in biochemical or molecular assays (Atlas, R.M., & Snyder, J.W. (2006)

6.4.2 Extraction crude metabolites

This process involves cultivating fungal strains in a nutrient-rich liquid medium under controlled conditions, typically for 7 to 14 days, to promote the production of secondary metabolites (Rani & Prasad, 2021). Post-incubation, the fungal biomass is separated from the culture broth through filtration or centrifugation (Mahfur et al., 2023). The resulting filtrate, containing extracellular metabolites, is subjected to solvent extraction using organic solvents such as ethyl acetate, methanol, or chloroform (Nickles et al., 2021). This step facilitates the transfer of bioactive compounds into the organic phase (Mahfur et al., 2023). The solvent layer is then concentrated using rotary evaporation or similar techniques to obtain the crude extract (Nickles et al., 2021).

Materials required

For Fungal Cultivation:

- Funnel
- Centrifuge system
- Falcons
- Whatman paper
- Erlenmeyer's flasks
- Ethanol (70%)
- Cotton
- Label and marker
- Tissue paper
- Shaking incubator (100–150 rpm)
- Fungal strain (from PDA slant or plate)
- Potato Dextrose Broth (PDB)

For Extraction:

- Centrifuge or filtration setup (Whatman filter paper, Buchner funnel)
- Separating funnel (500 mL or 1 L)
- Organic solvents (e.g., ethyl acetate, methanol, chloroform, acetone)
- Rotary evaporator or water bath for solvent evaporation
- Sterile glass beakers and measuring cylinders
- Glass vials or bottles for extract collection
- Mortar and pestle or tissue homogenizer (if extracting from biomass)
- Personal protective equipment (PPE: gloves, lab coat, goggles)
- Fume hood (for safe solvent handling)
- Labels and marker pens.

Procedure

1. The 21 days inoculated fungal cultures of alligator weed on PDB was taken out from incubator ((Kumar et al., 2019).
2. The fungal cultures are distributed equally in the following falcons respectively marked according to their strain name along with their replicas present ((Zhao & Mehta, 2020).
3. Then the above-mentioned falcons containing alligator weed were centrifuged at 6000 rpm for 15 minutes to make it cell- free (Singh et al., 2018).

4. The supernatant was collected by setting Whatman paper into the funnel through the process of filtration and is collected in a Erlenmeyer's flask ((Patel & Roy, 2021).
5. The above process is performed again for each of the different strains to obtain a cell-free metabolite ((Ahmed et al., 2022).

6.4.3 Toxicity test for extracted crude metabolites

To explore the possible phytotoxic and biotoxic impacts of crude, cell-free fungal metabolite extracts derived from alligator weed, we will analyse their effects on the germination, growth, and survival of specific test organisms in a controlled laboratory setting (Singh et al., 2021).

Materials required

• Whole plant of alligator weed
• Tap water
• Glass beakers
• Plastic covers
• Spray bottles
• Ethanol (70%)
• Distilled water

PROCEDURE

1. Healthy, uniform alligator weed plants or cuttings are collected and grown in pots or trays under greenhouse or growth chamber conditions (25–30°C, 60–70% RH, and 12 h photoperiod) to ensure acclimatization before treatment ((Dutta et al., 2024; Smith & Johnson, 2018)
2. Prepared multiple replicates for each treatment group to ensure statistical validity and account for biological variability (Brown et al., 2019; ResearchGate Protocol, 2022)
3. Thoroughly cleaned spray bottles by rinsing with ethanol followed by distilled water to ensure sterilization ((Green et al., 2017; ResearchGate Protocol, 2022)
4. Then Assigned a separate sterilized spray bottle for each fungal strain's metabolites ((Brown et al., 2019)
5. Filled each bottle with the respective fungal metabolites ((Dutta et al., 2024; Smith & Johnson, 2018).
6. Spraying the metabolites onto the alligator leaves until they are completely drenched.
7. Each setup was covered with plastic to retain moisture, creating a conducive environment for fungal activity.

8. The spraying procedure was carried out at intervals of 5 days, specifically on days 1, 5, 10, and 1((Brown et al., 2019; ResearchGate Protocol, 2022)
9. Observations were recorded on each of these days, noting any symptoms or changes in the plant ((Dutta et al., 2024; Smith & Johnson, 2018)
10. Monitored and documented the following symptoms to assess the impact of the fungal metabolites: Leaf Discoloration Look for browning or yellowing of leaves (Green et al., 2017). Tissue Damage: Identify signs of necrosis or blight (Smith & Johnson, 2018). Structural Changes: Note any wilting, curling, or collapse of leaf tissues ((Dutta et al., 2024). Progression: Track the spread and severity of symptoms over time ((Brown et al., 2019)

6.4.4 Statistical Analysis

To assess how fungal pathogens and their metabolites affect alligator weed (*Alternanthera philoxeroides*), a systematic statistical approach is followed. Data from experiments—such as measurements of weed biomass reduction, chlorophyll degradation, and mortality rates—are analysed using **analysis of variance (ANOVA)**. This method determines whether there are significant differences between various treatment groups in the study (Gomez & Gomez, 1984). Once significant effects are identified **post-hoc** tests are applied. These comparisons help identify which treatments, such as specific fungal isolates or metabolite doses, produce the most pronounced effects on the target weed (Zar, 1999).

All analyses are carried out using statistical software SPSS, which ensures precision, consistency, and replicability. This statistical workflow is essential for understanding the effectiveness of fungal-based biocontrol agents and their potential use in environmentally friendly weed management practices.

6.4.5 Morphological Identification of the Infecting Fungal Species

Materials Required

- Fungal culture isolates **(from infected alligator weed leaves)**
- Lactophenol cotton blue (as the mounting medium)
- Microscope slides and cover slips
- Sterile distilled water
- Glass slides (for temporary mounting of leaf samples)
- Ethanol (70%) (for surface sterilization)
- Tissue paper or blotting paper (for drying excess moisture)

- Gloves and lab coat (for safety and hygiene)
- Zeiss Axioscope A1 microscope or equivalent light microscope
- Clean sterile cello tapes.

Procedure

- Fungal isolates recovered from infected leaves of *Alternanthera philoxeroides* were grown on Potato Dextrose Agar (PDA) and incubated at 25–28°C for 5–7 days to promote sporulation (Gilman, 1959).
- A small piece (approximately 2–3 cm) of transparent adhesive tape (Scotch tape) was gently pressed onto the surface of the fungal colony to collect reproductive structures such as conidia and conidiophores. Care was taken to apply minimal pressure to preserve the natural arrangement of the structures (Barnett & Hunter, et al., 1960).
- One to two drops of lactophenol cotton blue were placed on a clean microscope slide to stain and fix the fungal elements (Ellis, 1971).
- The tape, carrying fungal material on its adhesive surface, was carefully placed sticky side down onto the stain on the slide extra stain was wiped using tissue paper. The tape was pressed gently to remove air bubbles and ensure a clear microscopic view (Ellis, 1976).
- The prepared slides were observed under a Zeiss Axioscope A1 compound microscope at 10×, 40×, and 100× magnifications. Key morphological characteristics—such as spore shape, size, septation, pigmentation, and arrangement—were recorded (Gilman, 1959; Barnett & Hunter, 1960).
- The fungal isolates were identified to genus level, and where possible to species level, based on comparative analysis with morphological descriptions in standard taxonomic literature (Gilman, 1959; Barnett & Hunter, 1960; Ellis, 1971; Ellis, 1976).

CHAPTER 07: RESULTS AND DISCUSSIONS

7.1 Disease Survey and Isolation of Pathogenic Fungi

Samples of diseases alligator weed were collected during the field surveys in Kolkata and adjacent areas, including North 24 Parganas, South 24 Parganas, Howrah and Birbhum, West Bengal, India, from January 2025 to March, 2025. Most of the diseased leaves showed leaf spots and chlorotic and necrotic lesions on the surface of the leaves. Fifteen fungal isolates (ALI-2 ALI-3, ALI-4, ALI-5, ALI-6, ALI-7, ALI-8, ALI-9, ALI-10, ALI-11, ALI-12, ALI-13, ALI-14, ALI -15, ALI 16) lab accession numbers were successfully isolated and purified from the diseased alligator leaves from the above- stated survey sites. The findings of this study emphasize the need for continuous monitoring of pathogenic fungi in agricultural settings. Implementing effective disease management strategies, including crop rotation, resistant cultivars, and appropriate fungicide application, is crucial to mitigate the impact of these pathogens on crop yield and quality.

FIG 7.1: - Diseased leaves of alligator weed

7.2 Inoculation of diseased leaves on Potato Dextrose agar

The inoculated plates of diseased leaves of water hyacinth in Potato Dextrose Agar showed different fungal colonies on each of the following inoculated plates, that was observed after 3-5 days after the incubated

7.3 Sub cultured to obtain pure culture

Obtained pure culture, derived from subculturing a single colony containing only one type of microorganisms making it ideal for studies, accurate identification, characteristics and their behaviour. It is done to maintain purity and viability of fungal culture. It helps in isolating specific colonies for identification or further study.

Subculturing for isolation of pure fungal culture

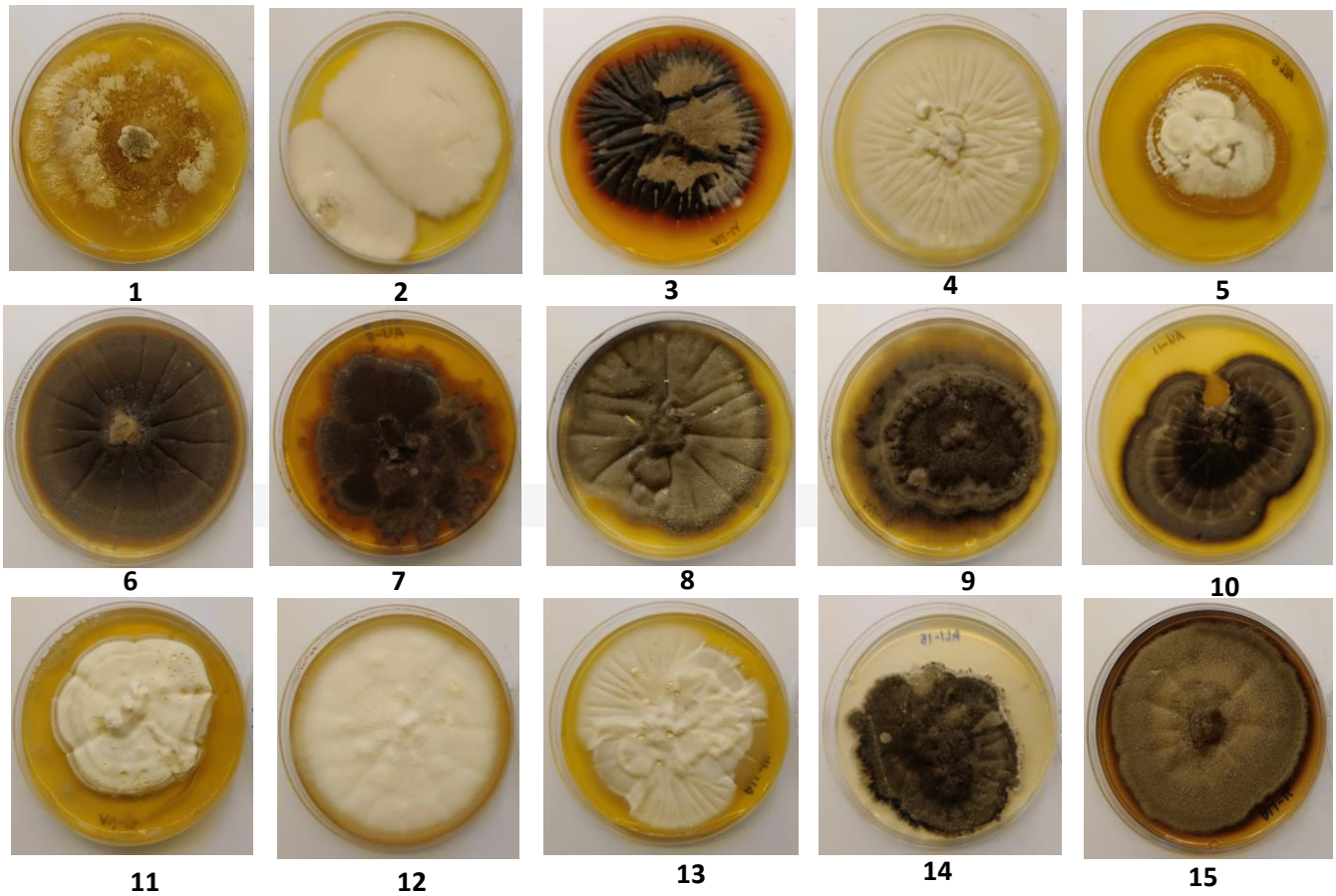


FIG 7.3.3: Fungal mother plates with lab accession (ALI 1 ALI 2 ALI 3 ALI 4 ALI 5 ALI 6 ALI 7 ALI 8 ALI 9 ALI 10 ALI 11 ALI 12 ALI 13 ALI 14 ALI 15 ALI 16) showing initial growth, colony morphology.

7.4 Toxicity test of Alligator weed

The primary objective in assessing the toxicity levels of alligator weed (*Alternanthera philoxeroides*) is to determine the concentration of its bioactive compounds and understand their potential effects on various organisms. This includes evaluating the risks posed to livestock through heavy metal accumulation and understanding the plant's allelopathic effects on native vegetation. Among 15 isolates, all the fifteen (ALI-2 ALI-3, ALI-4, ALI-5, ALI-6, ALI-7, ALI-8, ALI-9, ALI-10, ALI-11, ALI-12, ALI-13, ALI-14, ALI -15, ALI-16) caused severe leaf damage, within 5 days of application. While the control showed no or negligible disease. The 15 isolates showing promising results in toxicity assay were selected for whole plant assay.

CONTROL

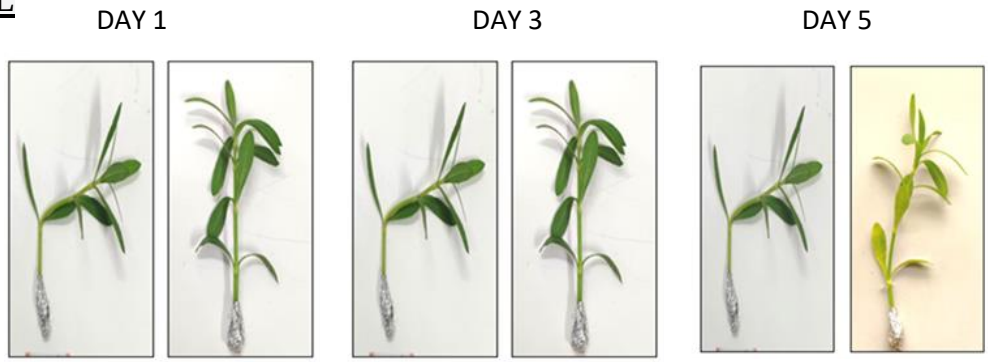


FIG: - Control leaves treated with di. H_2O and Tween 20 solution respectively

ALI-2

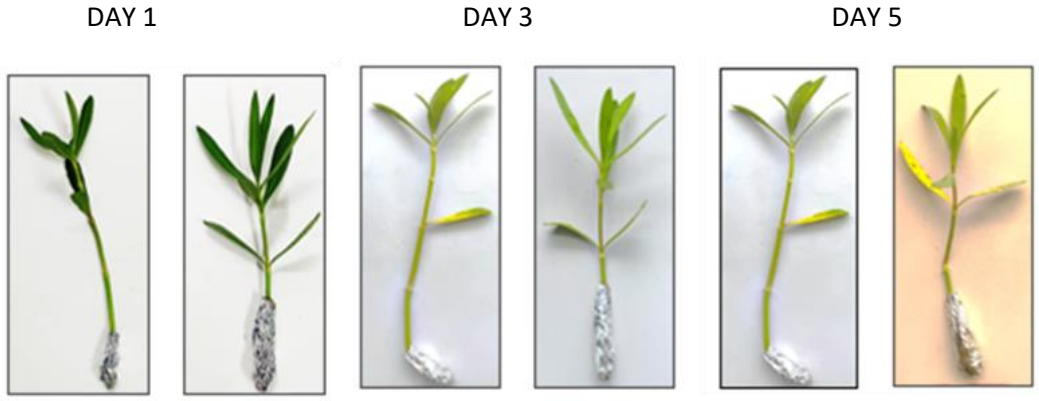


Figure 7.4.1 | Toxicity assay of fungus (ALI-2) observed on day 01 after application of fungus (AL2) on the healthy leaves of water hyacinth. A| Fungus (ALI1) with distilled water, B| Fungus (ALI-2) with Tween20

Figure 7.4.2 | Toxicity assay of fungus (ALI-2) observed on day 03 after application of fungus (ALI-2) on the healthy leaves of water hyacinth. A| Fungus (ALI 2) with distilled water, B| Fungus (ALI-2) with Tween20 solution

Figure 7.4.3 | Toxicity assay of fungus (ALI-2) observed on day 05 after application of fungus (ALI-2) on the healthy leaves of water hyacinth. A| Fungus (ALI 2) with distilled water, B| Fungus (ALI-2) with Tween20 solution

ALI-3

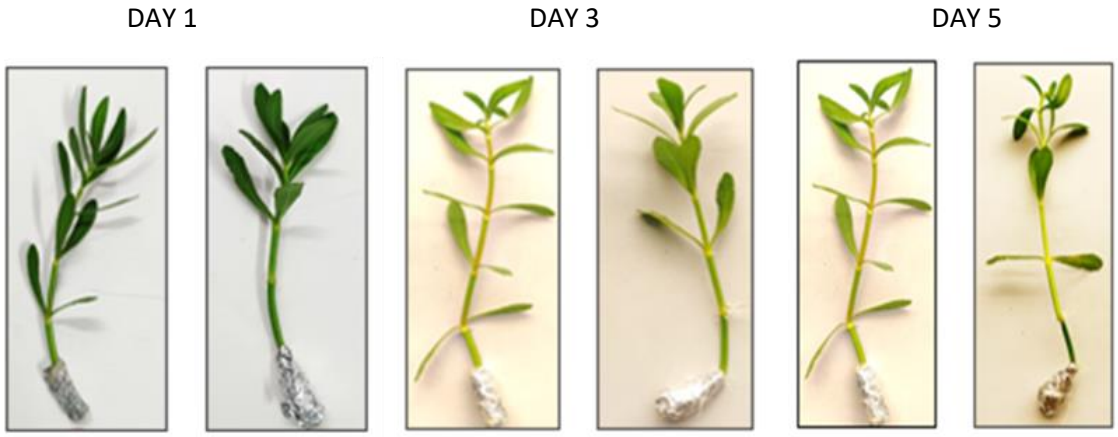


Figure 7.4.4 | Toxicity assay of fungus (ALI-3) observed on day 01 after application of fungus (ALI 3) on the healthy leaves of water hyacinth. A| Fungus (ALI 3) with distilled water, B| Fungus (ALI-3) with Tween20 solution

Figure 7.4.5 | Toxicity assay of fungus (ALI-3) observed on day 03 after application of fungus (ALI 3) on the healthy leaves of water hyacinth. A| Fungus (ALI 3) with distilled water, B| Fungus (ALI-3) with Tween20 solution

Figure 7.4.6 | Toxicity assay of fungus (ALI-3) observed on day 05 after application of fungus (ALI 3) on the healthy leaves of water hyacinth. A| Fungus (ALI 3) with distilled water, B| Fungus (ALI-3) with Tween20 solution

ALI-4

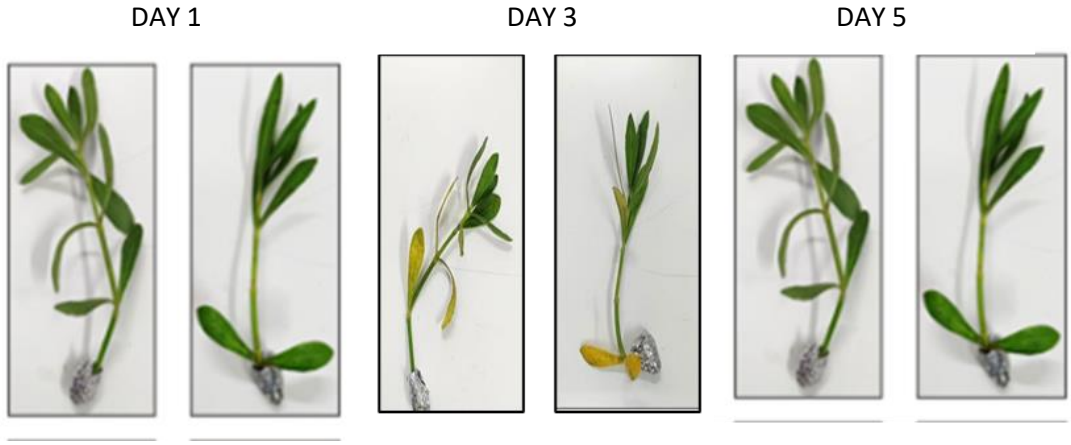


Figure 7.4.7 | Toxicity assay of fungus (ALI-4) observed on day 01 after application of fungus (ALI 4) on the healthy leaves of water hyacinth. A| Fungus (ALI 4) with distilled water, B| Fungus (ALI-4) with Tween20 solution

Figure 7.4.8 | Toxicity assay of fungus (ALI-4) observed on day 03 after application of fungus (ALI 4) on the healthy leaves of water hyacinth. A| Fungus (ALI 4) with distilled water, B| Fungus (ALI-4) with Tween20 solution

Figure 7.4.9 | Toxicity assay of fungus (ALI-4) observed on day 05 after application of fungus (ALI 4) on the healthy leaves of water hyacinth. A| Fungus (ALI 4) with distilled water, B| Fungus (ALI-4) with Tween20 solution

ALI-5

DAY 1

DAY 3

DAY 5

Figure 7.4.10 | Toxicity assay of fungus (ALI-5) observed on day 01 after application of fungus (ALI-5) on the healthy leaves of water hyacinth. A| Fungus (ALI-5) with distilled water, B| Fungus (ALI-5) with Tween20 solution

Figure 7.4.11 | Toxicity assay of fungus (ALI-5) observed on day 03 after application of fungus (ALI-5) on the healthy leaves of water hyacinth. A| Fungus (ALI-5) with distilled water, B| Fungus (ALI-5) with Tween20 solution

Figure 7.4.12 | Toxicity assay of fungus (ALI-5) observed on day 05 after application of fungus (ALI-5) on the healthy leaves of water hyacinth. A| Fungus (ALI-5) with distilled water, B| Fungus (ALI-5) with Tween20 solution

ALI-6

DAY 1

DAY 3

DAY 5

Figure 7.4.13 | Toxicity assay of fungus (ALI-6) observed on day 01 after application of fungus (ALI-6) on the healthy leaves of water hyacinth. A| Fungus (ALI-6) with distilled water, B| Fungus (ALI-6) with Tween20 solution

Figure 7.4.14 | Toxicity assay of fungus (ALI-6) observed on day 03 after application of fungus (ALI-6) on the healthy leaves of water hyacinth. A| Fungus (ALI-6) with distilled water, B| Fungus (ALI-6) with Tween20 solution

Figure 7.4.15 | Toxicity assay of fungus (ALI-6) observed on day 05 after application of fungus (ALI-6) on the healthy leaves of water hyacinth. A| Fungus (ALI-6) with distilled water, B| Fungus (ALI-6) with Tween20 solution

ALI-7

DAY 1

Figure 7.4.16 | Toxicity assay of fungus (ALI-7) observed on day 01 after application of fungus (ALI-7) on the healthy alligator weed. A| Fungus (ALI-7) with distilled water, B| Fungus (ALI-7) with Tween20 solution

DAY 3

Figure 7.4.17 | Toxicity assay of fungus (ALI-7) observed on day 03 after application of fungus (ALI-7) on the healthy alligator weed. A| Fungus (ALI-7) with distilled water, B| Fungus (ALI-7) with Tween20 solution

DAY 5

Figure 7.4.18 | Toxicity assay of fungus (ALI-7) observed on day 05 after application of fungus (ALI-7) on the healthy alligator weed. A| Fungus (ALI-7) with distilled water, B| Fungus (ALI-7) with Tween20 solution

ALI-8

DAY 1

Figure 7.4.19 | Toxicity assay of fungus (ALI-8) observed on day 01 after application of fungus (ALI-8) on the healthy alligator weed. A| Fungus (ALI-8) with distilled water, B| Fungus (ALI-8) with Tween20 solution

DAY 3

Figure 7.4.20 | Toxicity assay of fungus (ALI-8) observed on day 03 after application of fungus (ALI-8) on the healthy alligator weed. A| Fungus (ALI-8) with distilled water, B| Fungus (ALI-8) with Tween20 solution

DAY 5

Figure 7.4.21 | Toxicity assay of fungus (ALI-8) observed on day 05 after application of fungus (ALI-8) on the healthy alligator weed. A| Fungus (ALI-8) with distilled water, B| Fungus (ALI-8) with Tween20 solution

ALI-9

DAY 1

Figure 7.4.22 | Toxicity assay of fungus (ALI-9) observed on day 01 after application of fungus (ALI-9) on the healthy alligator weed. A| Fungus (ALI-9) with distilled water, B| Fungus (ALI-9) with Tween20 solution

DAY 3

Figure 7.4.23 | Toxicity assay of fungus (ALI-9) observed on day 03 after application of fungus (ALI-9) on the healthy alligator weed. A| Fungus (ALI-9) with distilled water, B| Fungus (ALI-9) with Tween20 solution

DAY 5

Figure 7.4.24 | Toxicity assay of fungus (ALI-9) observed on day 05 after application of fungus (ALI-9) on the healthy alligator weed. A| Fungus (ALI-9) with distilled water, B| Fungus (ALI-9) with Tween20 solution

ALI-10

DAY 1

Figure 7.4.25 | Toxicity assay of fungus (ALI-10) observed on day 01 after application of fungus (ALI-10) on the healthy alligator weed. A| Fungus (ALI-10) with distilled water, B| Fungus (ALI-10) with Tween20 solution

DAY 3

Figure 7.4.26 | Toxicity assay of fungus (ALI-10) observed on day 03 after application of fungus (ALI-10) on the healthy alligator weed. A| Fungus (ALI-10) with distilled water, B| Fungus (ALI-10) with Tween20 solution

DAY 5

Figure 7.4.27 | Toxicity assay of fungus (ALI-10) observed on day 05 after application of fungus (ALI-10) on the healthy alligator weed. A| Fungus (ALI-10) with distilled water, B| Fungus (ALI-10) with Tween20 solution

ALI 11

DAY 1

Figure 7.4.28 | Toxicity assay of fungus (ALI-11) observed on day 01 after application of fungus (ALI-11) on the healthy alligator weed. A| Fungus (ALI-11) with distilled water, B| Fungus (ALI-11) with Tween20 solution

DAY 3

Figure 7.4.29 | Toxicity assay of fungus (ALI-11) observed on day 03 after application of fungus (ALI-11) on the healthy alligator weed. A| Fungus (ALI-11) with distilled water, B|Fungus (ALI-11) with Tween20 solution

DAY 5

Figure 7.4.30 | Toxicity assay of fungus (ALI-11) observed on day 05 after application of fungus (ALI-11) on the healthy alligator weed. A| Fungus (ALI-11) with distilled water, B|Fungus (ALI-11) with Tween20 solution

ALI 12

DAY 1

Figure 7.4.31 | Toxicity assay of fungus (ALI-12) observed on day 01 after application of fungus (ALI-12) on the healthy alligator weed. A| Fungus (ALI-12) with distilled water, B| Fungus (ALI-12) with Tween20 solution

DAY 3

Figure 7.4.32 | Toxicity assay of fungus (ALI-12) observed on day 03 after application of fungus (ALI-12) on the healthy alligator weed. A| Fungus (ALI-12) with distilled water, B| Fungus (ALI-12) with Tween20 solution

DAY 5

Figure 7.4.33 | Toxicity assay of fungus (ALI-12) observed on day 05 after application of fungus (ALI-12) on the healthy alligator weed. A| Fungus (ALI-12) with distilled water, B| Fungus (ALI-12) with Tween20 solution

ALI 13

DAY 1

Figure 7.4.34 | Toxicity assay of fungus (ALI-13) observed on day 01 after application of fungus (ALI-13) on the healthy alligator weed. A| Fungus (ALI-13) with distilled water, B| Fungus (ALI-13) with Tween20 solution

DAY 3

Figure 7.4.35 | Toxicity assay of fungus (ALI-13) observed on day 01 after application of fungus (ALI-13) on the healthy alligator weed. A| Fungus (ALI-13) with distilled water, B| Fungus (ALI-13) with Tween20 solution

DAY 5

Figure 7.4.36 | Toxicity assay of fungus (ALI-13) observed on day 01 after application of fungus (ALI-13) on the healthy alligator weed. A| Fungus (ALI-13) with distilled water, B| Fungus (ALI-13) with Tween20 solution

ALI 14

DAY 1

Figure 7.4.37 | Toxicity assay of fungus (ALI-14) observed on day 01 after application of fungus (ALI-14) on the healthy alligator weed. A| Fungus (ALI-14) with distilled water, B| Fungus (ALI-14) with Tween20 solution

DAY 3

Figure 7.4.38 | Toxicity assay of fungus (ALI-14) observed on day 01 after application of fungus (ALI-14) on the healthy alligator weed. A| Fungus (ALI-14) with distilled water, B| Fungus (ALI-14) with Tween20 solution

DAY 5

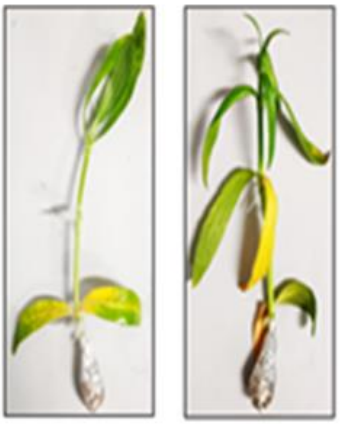


Figure 7.4.39 | Toxicity assay of fungus (ALI-14) observed on day 01 after application of fungus (ALI-14) on the healthy alligator weed. A| Fungus (ALI-14) with distilled water, B| Fungus (ALI-14) with Tween20 solution

ALI-15

DAY 1

Figure 7.4.40 | Toxicity assay of fungus (ALI-15) observed on day 01 after application of fungus (ALI-15) on the healthy alligator weed. A| Fungus (ALI-15) with distilled water, B| Fungus (ALI-15) with Tween20 solution

DAY 3

Figure 7.4.41 | Toxicity assay of fungus (ALI-15) observed on day 03 after application of fungus (ALI-15) on the healthy alligator weed. A| Fungus (ALI-15) with distilled water, B| Fungus (ALI-15) with Tween20 solution

DAY 5

Figure 7.4.42 | Toxicity assay of fungus (ALI-15) observed on day 05 after application of fungus (ALI-15) on the healthy alligator weed. A| Fungus (ALI-15) with distilled water, B| Fungus (ALI-15) with Tween20 solution

ALI-16

DAY 1

Figure 7.4.43 | Toxicity assay of fungus (ALI-16) observed on day 01 after application of fungus (ALI-16) on the healthy alligator weed. A| Fungus (ALI-16) with distilled water, B| Fungus (ALI-16) with Tween20 solution

DAY 3

Figure 7.4.44 | Toxicity assay of fungus (ALI-16) observed on day 03 after application of fungus (ALI-16) on the healthy alligator weed. A| Fungus (ALI-16) with distilled water, B| Fungus (ALI-16) with Tween20 solution

DAY 5

Figure 7.4.45 | Toxicity assay of fungus (ALI-16) observed on day 05 after application of fungus (ALI-16) on the healthy alligator weed. A| Fungus (ALI-16) with distilled water, B| Fungus (ALI-16) with Tween20 solution

Strain Number	Day 01	Day 03	Day 05
ALI - 2 + dH_2O	No disease	Mild	Mild
ALI - 2 + Tween 20	No disease	Mild	Severe
ALI - 3 + dH_2O	No disease	No disease	Mild
ALI - 3 + Tween 20	No disease	Mild	Severe
ALI - 4 + dH_2O	No disease	Mild	Severe
ALI - 4 + Tween 20	No disease	Mild	Severe
ALI - 5 + dH_2O	No disease	Mild	Mild
ALI - 5 + Tween 20	No disease	Mild	Severe
ALI - 6 + dH_2O	No disease	No disease	Mild
ALI - 6 + Tween 20	No disease	Mild	Severe
ALI - 7 + dH_2O	No disease	Mild	Mild
ALI - 7 + Tween 20	No disease	Mild	Severe
ALI - 8 + dH_2O	No disease	Mild	Mild
ALI - 8 + Tween 20	No disease	Severe	Severe
ALI - 9 + dH_2O	No disease	Mild	Severe
ALI - 9 + Tween 20	No disease	Mild	Severe
ALI - 10 + dH_2O	No disease	Mild	Severe
ALI - 10 + Tween 20	No disease	Severe	Severe
ALI - 11 + dH_2O	No disease	Mild	Mild
ALI - 11 + Tween 20	No disease	Mild	Severe
ALI - 12 + dH_2O	No disease	Mild	Severe
ALI - 12 + Tween 20	No disease	Mild	Severe
ALI - 13 + dH_2O	No disease	Mild	Mild

CONTINUED

Strain Number	Day 01	Day 03	Day 05
ALI - 13 + Tween 20	No disease	Mild	Mild
ALI - 14 + dH_2O	No disease	Mild	Mild
ALI - 14 + Tween 20	No disease	Mild	Severe
ALI - 15 + dH_2O	Mild	Severe	Severe
ALI - 15 + Tween 20	Mild	Severe	Severe
ALI - 16 + dH_2O	No disease	Mild	Mild
ALI - 16 + Tween 20	No disease	Mild	Mild

Table 7.1: - The progression of disease severity over time for each strain under two different treatments: dH_2O and Tween 20 showed in tabular form.

ABBREVIATIONS:

- dH_2O = distilled water

7.5 Whole plant bioassay

Out of 15 isolates, five isolates (ALI-2, ALI-4, ALI-10, ALI-14, ALI -15) caused severe diseased with almost ≥50% foliar damage when tested in the whole plant pathogenicity test, whereas the remaining isolates (ALI-3, ALI-5, ALI-6, ALI-7, ALI-8, ALI-9, ALI -11, ALI-12, ALI-13, ALI-16)) damaged plant leaves around foliar damage percentage < 50%, but did not result in plant death.

CONTROL

FIG 7.5.1: - Untreated control plant showing normal growth

(ALI-2)

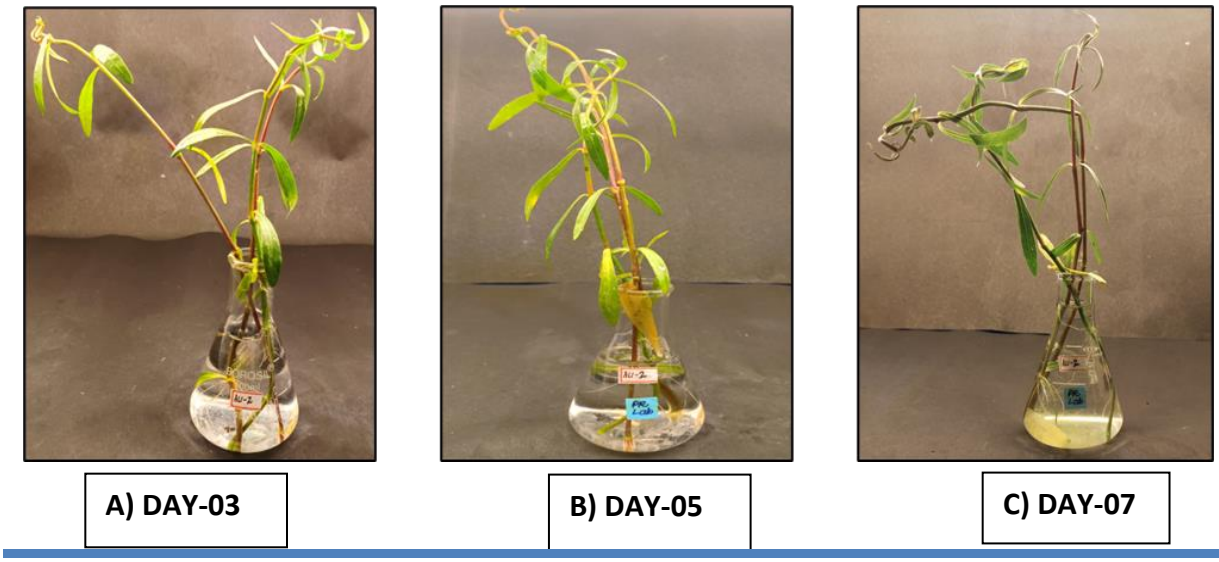


FIG 7.5.2: - Whole plant assay of fungus ALI-2, *A*| Healthy whole alligator weed Observed on day 3 after application of fungus with T20 solution *B*| Healthy whole alligator weed Observed on day 5 after application of fungus with T20 solution *C*| Healthy whole alligator weed Observed on day 7 after application of fungus with T20 solution

(ALI-3)

A) DAY-03 | B) DAY-05 | C) DAY-07

FIG 7.5.3: - Whole plant assay of fungus ALI-3, *A*| Healthy whole alligator weed Observed on day 3 after application of fungus with T20 solution *B*| Healthy whole alligator weed Observed on day 5 after application of fungus with T20 solution *C*| Healthy whole alligator weed Observed on day 7 after application of fungus with T20 solution

(ALI-4)

A) DAY-03 | B) DAY-05 | C) DAY-07

FIG 7.5.4: - Whole plant assay of fungus ALI-4, *A*/ Healthy whole alligator weed Observed on day 3 after application of fungus with T20 solution *B*/ Healthy whole alligator weed Observed on day 5 after application of fungus with T20 solution *C*/ Healthy whole alligator weed Observed on day 7 after application of fungus with T20 solution

(ALI-5)

A) DAY-03 B) DAY-05 C) DAY-07

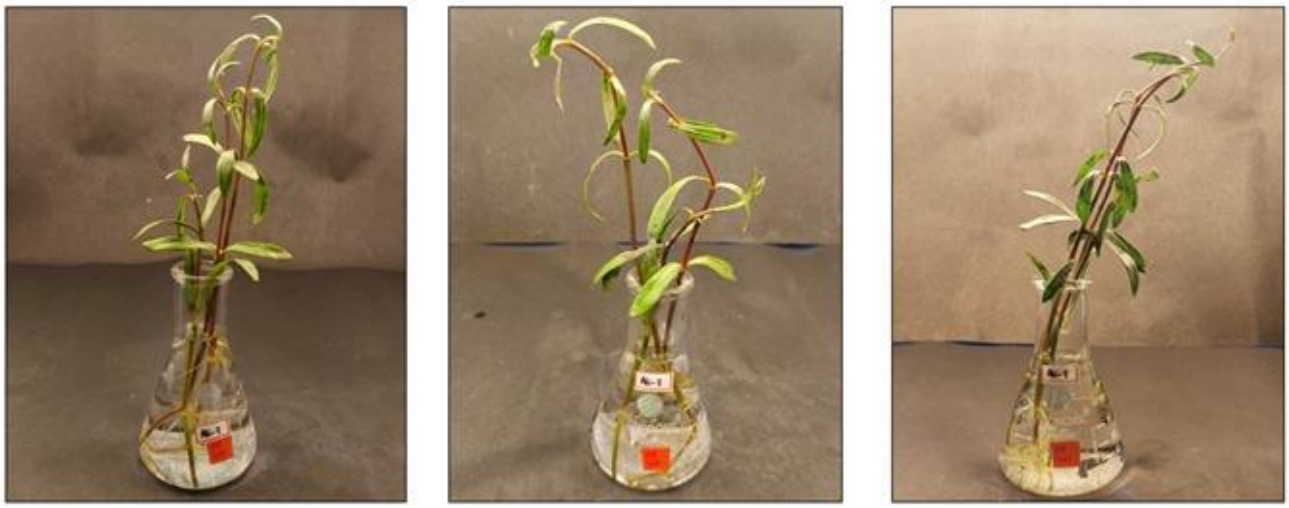
FIG 7.5.5: - Whole plant assay of fungus ALI-4, *A*/ Healthy whole alligator weed Observed on day 3 after application of fungus with T20 solution *B*/ Healthy whole alligator weed Observed on day 5 after application of fungus with T20 solution *C*/ Healthy whole alligator weed Observed on day 7 after application of fungus with T20 solution

(ALI-6)

A) DAY-03 B) DAY-05 C) DAY-07

FIG 7.5.6 : - Whole plant assay of fungus ALI-6, *A*/ Healthy whole alligator weed Observed on day 3 after application of fungus with T20 solution *B*/ Healthy whole alligator weed Observed on day 5 after application of fungus with T20 solution *C*/ Healthy whole alligator weed Observed on day 7 after app fungus with T20 solution

(ALI-7)



C) DAY-03 | B) DAY-03 | A) DAY-03

FIG 7.5.7: - Whole plant assay of fungus ALI-7, *A*/ Healthy whole alligator weed Observed on day 3 after application of fungus with T20 solution *B*/ Healthy whole alligator weed Observed on day 5 after application of fungus with T20 solution *C*/ Healthy whole alligator weed Observed on day 7 after application of fungus with T20 solution

(ALI-8)

D) DAY-03 | B) DAY-05 | C) DAY-07

FIG 7.5.8: - Whole plant assay of fungus ALI-8, *A*/ Healthy whole alligator weed Observed on day 3 after application of fungus with T20 solution *B*/ Healthy whole alligator weed Observed on day 5 after application of fungus with T20 solution *C*/ Healthy whole alligator weed Observed on day 7 after application of fungus with T20 solution

(ALI-9)

A) DAY-03

B) DAY-05

C) DAY-07

FIG 7.5.9: - Whole plant assay of fungus ALI-9, *A*/ Healthy whole alligator weed Observed on day 3 after application of fungus with T20 solution *B*/ Healthy whole alligator weed Observed on day 5 after application of fungus with T20 solution *C*/ Healthy whole alligator weed Observed on day 7 after application of fungus with T20 solution

(ALI-10)

A) DAY-03

B) DAY-05

C) DAY-07

FIG 7.5.10: - Whole plant assay of fungus ALI-10, *A*/ Healthy whole alligator weed Observed on day 3 after application of fungus with T20 solution *B*/ Healthy whole alligator weed Observed on day 5 after application of fungus with T20 solution *C*/ Healthy whole alligator weed Observed on day 7 after application of fungus with T20 solution

(ALI-11)

A) DAY-03 | B) DAY-05 | C) DAY-07

FIG 7.5.11: - Whole plant assay of fungus ALI-11, *A*/ Healthy whole alligator weed Observed on day 3 after application of fungus with T20 solution *B*/ Healthy whole alligator weed Observed on day 5 after application of fungus with T20 solution *C*/ Healthy whole alligator weed Observed on day 7 after application of fungus with T20 solution

(ALI-12)

A) DAY-03 | B) DAY-05 | C) DAY-07

FIG 7.5.12: - Whole plant assay of fungus ALI-12, *A*/ Healthy whole alligator weed Observed on day 3 after application of fungus with T20 solution *B*/ Healthy whole alligator weed Observed on day 5 after application of fungus with T20 solution *C*/ Healthy whole alligator weed Observed on day 7 after application of fungus with T20 solution

(ALI-13)

A) DAY-03 | B) DAY-05 | C) DAY-07

FIG 7.5.13: - Whole plant assay of fungus ALI-13, *A*/ Healthy whole alligator weed Observed on day 3 after application of fungus with T20 solution *B*/ Healthy whole alligator weed Observed on day 5 after application of fungus with T20 solution *C*/ Healthy whole alligator weed Observed on day 7 after application of fungus with T20 solution

(ALI-14)

A) DAY-03 | B) DAY-05 | C) DAY-07

FIG 7.5.14: - Whole plant assay of fungus ALI-14, *A*/ Healthy whole alligator weed Observed on day 3 after application of fungus with T20 solution *B*/ Healthy whole alligator weed Observed on day 5 after application of fungus with T20 solution *C*/ Healthy whole alligator weed Observed on day 7 after application of fungus with T20 solution

(ALI-15)

A) DAY-03 | B) DAY-05 | C) DAY-07

FIG 7.5.15: - Whole plant assay of fungus ALI-15, *A*/ Healthy whole alligator weed Observed on day 3 after application of fungus with T20 solution *B*/ Healthy whole alligator weed Observed on day 5 after application of fungus with T20 solution *C*/ Healthy whole alligator weed Observed on day 7 after application of fungus with T20 solution

ALI-(16)

A) DAY-03 | B) DAY-05 | C) DAY-07

FIG 7.5.16: - Whole plant assay of fungus ALI-16, *A*/ Healthy whole alligator weed Observed on day 3 after application of fungus with T20 solution *B*/ Healthy whole alligator weed Observed on day 5 after application of fungus with T20 solution *C*/ Healthy whole alligator weed Observed on day 7 after application of fungus with T20 solution

	DAY 03	DAY 05	DAY 07
STRAINS	**FD (%) Mean ±SE**	**FD (%) Mean ±SE**	**FD (%) Mean ±SE**
ALI 2	**8.333(±4.811)**	**25 (±4.811)**	**52.78(±2.778)**
ALI 3	**3.333(±3.333)**	**10(±5.774)**	**13.33(±8.819)**
ALI 4	**6.667(±3.333)**	**30(±5.774)**	**50(±5.774)**
ALI 5	**10(±5.774)**	**16.67(±3.333)**	**23.33(±6.667)**
ALI 6	**7.5(± 3.819)**	**29.17(±15.02)**	**32.5(±11.81)**
ALI 7	**0(±0)**	**0(±0)**	**4.167(±4.167)**
ALI 8	**0(±0)**	**10(±10)**	**40(±5.774)**
ALI 9	**6.667(±3.333)**	**19.17(±9.167)**	**35(±20.21)**
ALI 10	**9.444(±5.8)**	**31.11(±5.879)**	**53.33(±3.333)**
ALI 11	**10(5. ±774) ±**	**16.11(±3.889)**	**22.22(±4.006)**
ALI 12	**6.667(±3.333)**	**16.67(±6.667)**	**20(±5.774)**
ALI 13	**13.33(±6.667)**	**27.5(±11.46)**	**28.33(±1.667)**
ALI 14	**7.143(±4.124)**	**16.67(±13.26)**	**52.38(±6.299)**
ALI 15	**43.33(6 ±.667)**	**56.67(±12.02)**	**74.17(±8.207)**
ALI 16	**6.667(±6.667)**	**6.667(±6.667)**	**25.83(±7.949)**
CONTROL	**0(±0)**	**0(±0)**	**0(±0)**

Table 7.2: - FD (%) of whole plant bioassay of crude metabolites after 3,5,7 days

ABBREVIATIONS

- FD – Folliear damage
- SE- Standard error

ANOVA Table

			Sum of Squares	df	Mean Square	F	Sig.
FD (%) * STRAINS	Between Groups	(Combined)	4262.015	14	304.430	4.573	<.001
	Within Groups		1996.948	30	66.565		
	Total		6258.963	44			

DAY 3

ANOVA Table

			Sum of Squares	df	Mean Square	F	Sig.
FD (%) * STRAIN	Between Groups	(Combined)	7654.383	14	546.742	2.484	.018
	Within Groups		6603.959	30	220.132		
	Total		14258.342	44			

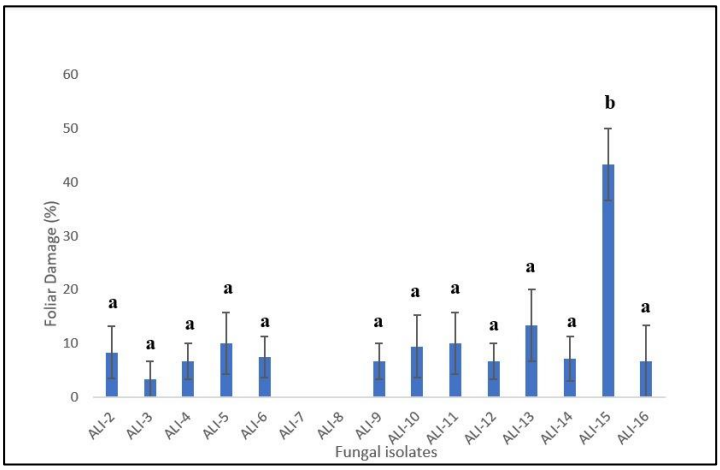
DAY 5

ANOVA Table

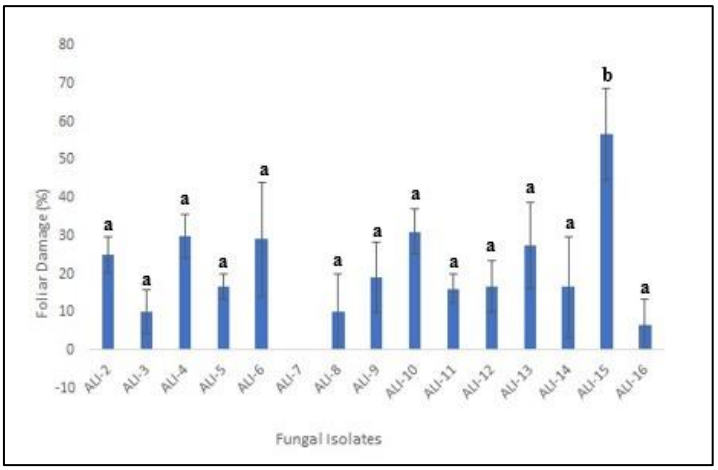
			Sum of Squares	df	Mean Square	F	Sig.
FD (%) * STRAINS	Between Groups	(Combined)	14451.455	14	1032.247	5.185	<.001
	Within Groups		5972.354	30	199.078		
	Total		20423.810	44			

DAY 7

Table 7.3: - ANOVA Table obtained by statistical analysis (SPSS) showing significant difference between variables.



DAY 03



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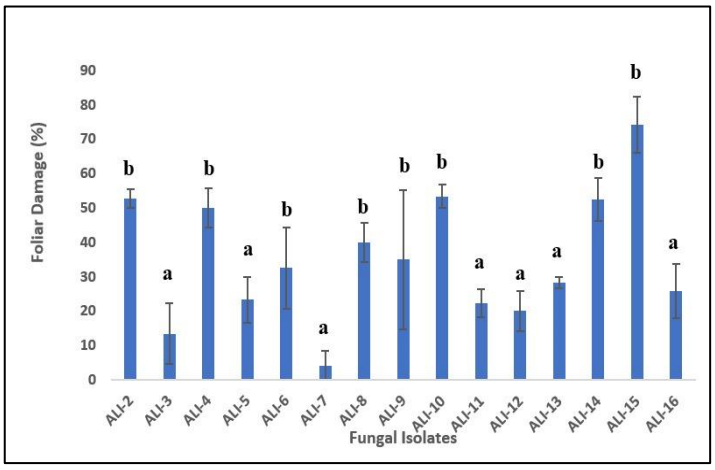


DAY 07

FIG 7.5.17: - Disease incidence on alligator weed after fungal inoculation in the whole plant pathogenicity assay 3, 5, 7 days after inoculation. Values are means and standard errors based on three replicates. Means marked with the same letter(s) above the bars are not significantly different according to bon Ferroni test ($p < 0.05$).

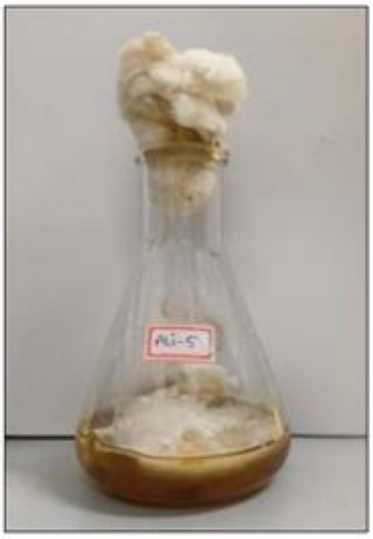
7.6 Crude metabolite extraction from fungal strain for bioactivity analysis`s

Crude metabolite test was performed to evaluate the toxic effects of crude metabolite on a selected biological system.

ALI 2

ALI 3

ALI 4



ALI 5

ALI 6

ALI 7

FIG 7.6.1: - Crude metabolite extraction from fungal isolate cultured in liquid medium of strains (ALI 2 ALI 3 ALI 4 ALI 5 ALI 6 ALI 7)

ALI 8

ALI 9

ALI 10

ALI 11

ALI 12

ALI 13

ALI 14

ALI 15

ALI 16

FIG 7.6.2: - Application of metabolite spray on whole plant to assess its phytotoxic effects.

7.7 Toxicity Test of Crude Metabolite

Sequential spraying of fungal metabolite on alligator weed for phytotoxicity study (day 5, day 10, day 15) Out of 15 fungal isolates evaluated crude metabolites from 5 strains (ALI 2, ALI 4, ALI 10, ALI 14, ALI 15) Demonstrated significant phytotoxicity against alligator weed at 5, 10, 15-day post treatment intervals.

DAY 5 **DAY 10** **DAY 15**

CONTROL **CONTROL** **CONTROL**

ALI 2 **ALI 2** **ALI 2**

ALI 4 **ALI 4** **ALI 4**

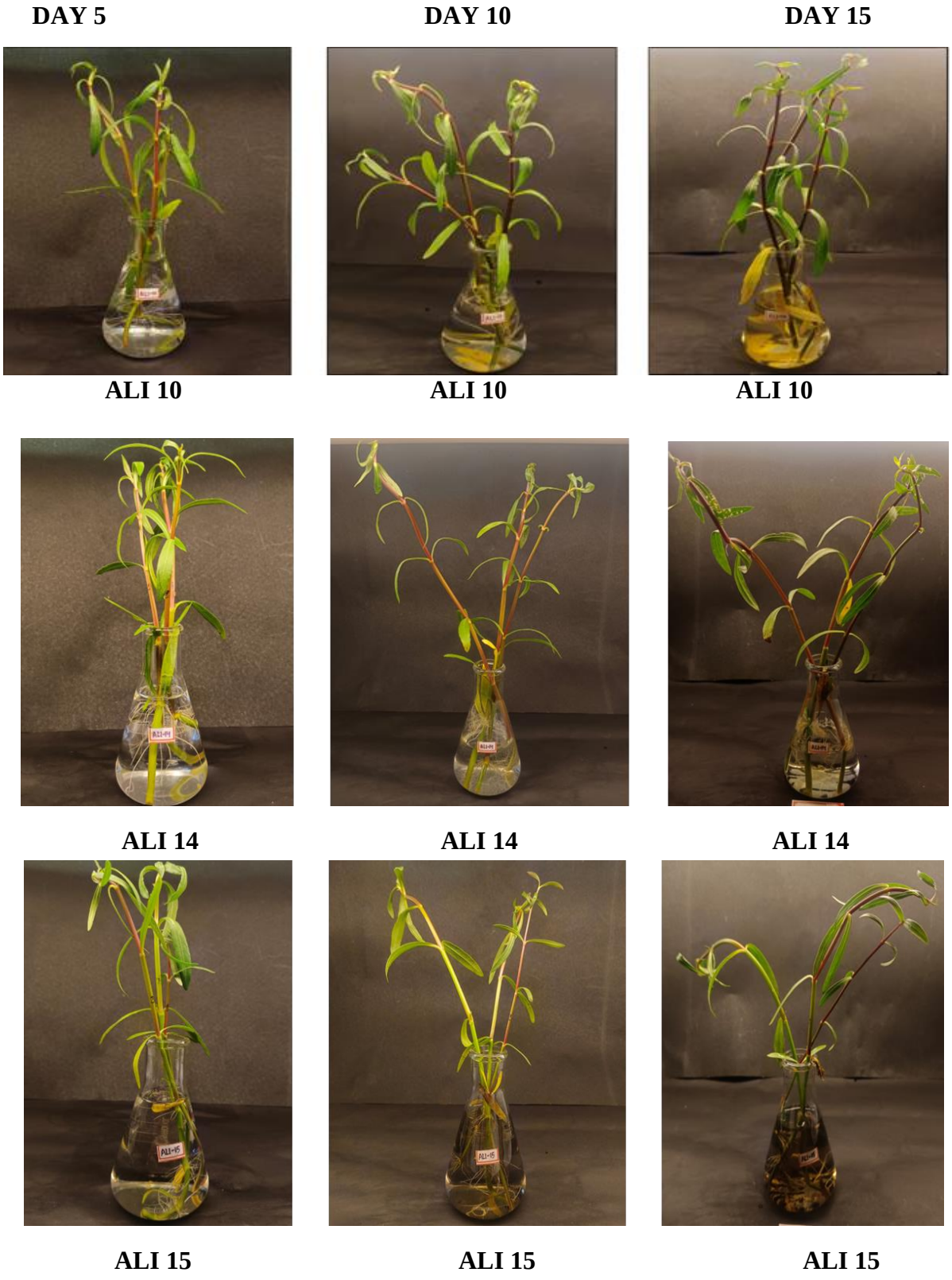


FIG 7.7: - Application of metabolite spray on whole plant to assess its phytotoxic effects.

Strain	FD (%) Mean ± SE
ALI 2	75.55 ± 4.44
ALI 4	38.88 ± 1.38
ALI 10	17.22 ± 4.33
ALI 14	13.61 ± 3.41
ALI 15	18.33 ± 4.40

Table 7.4: - FD (%) of toxicity test of crude metabolites after 15 days

ANOVA Table

			Sum of Squares	df	Mean Square	F	Sig.
FD(%) * STRAIN	Between Groups	(Combined)	4780.370	4	1195.093	4.013	.034
	Within Groups		2978.241	10	297.824		
	Total		7758.611	14			

Table 7.5: - ANOVA Table obtained by statistical analysis (SPSS) showing significant difference between variables for metabolite spray on day 15

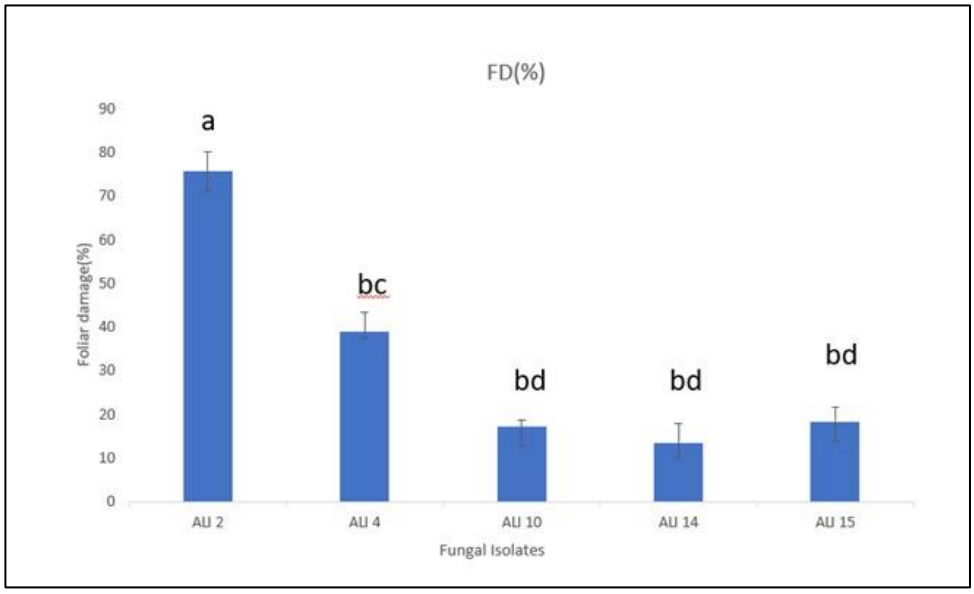


FIG 7.7.2: -Disease incidence on alligator weed after metabolite spraying in the whole plant pathogenicity assay after 15 days. Values are means and standard errors based on three replicates. Means marked with the same letter(s) above the bars are not significantly different according to bon Ferroni test (p< 0.05).

7.8 Morphological Identification

FIG 7.8: - Morphological characteristics of *Alternaria* species showing typical colony appearance and spore structures under the microscope.

After successfully identification of morphological strain, it was found that ALI 2 is *Alternaria* Sp.

CHAPTER 08: SIGNIFICANCE OF THE STUDY

1. Bioherbicides Developed from Fungi Provide a Sustainable Substitute for Synthetic Chemical Herbicides

Bioherbicides developed from fungi provide a sustainable substitute for synthetic chemical herbicides, tackling challenges like environmental degradation and biodiversity reduction. The exploration of fungal biocontrol agents focuses on identifying fungi capable of effectively suppressing weed growth under practical agricultural conditions. By targeting fungi that produce phytotoxic metabolites, researchers aim to develop bioherbicides that are both efficient and environmentally sustainable. For example, the bioherbicide Kichawi Kill™, formulated from Fusarium oxysporum f. sp., has been utilized to control the parasitic weed Striga hermonthica in Kenyan maize fields, resulting in substantial yield improvements (Avedi et al., 2014; Marley et al., 2004). This example underscores the potential of fungal agents in creating sustainable weed management solutions suitable for field application.

2. Fungal Metabolites as Eco-Friendly Alternatives to Chemical Herbicides

Fungal-derived bioherbicides present a promising alternative to synthetic chemical herbicides, addressing concerns related to environmental pollution and biodiversity loss. Fungi produce a diverse array of secondary metabolites, including polyketides, terpenoids, and nitrogenous compounds, which exhibit phytotoxic properties capable of inhibiting weed growth (Vurro & Evidente, 2007). Utilizing these natural compounds can reduce the reliance on chemical herbicides, thereby mitigating the adverse effects associated with their use, such as soil degradation and harm to non-target organisms (Harding & Raizada, 2015). Moreover, the specificity of certain fungal metabolites allows for targeted weed control, minimizing collateral damage to crops and beneficial plant species (Hoagland, 2001).

3. Identifying Promising Fungi and Their Bioactive Molecules for Further Research

The identification of fungi that produce potent bioactive compounds is crucial for advancing bioherbicide development. Species such as Trichoderma koningii and Trichoderma harzianum have shown significant potential in controlling various weed species (Howell, 2003). For example, T. koningii produces metabolites like trichokonins and koninginins, which exhibit phytotoxic properties against certain weeds (Dennis & Webster, 1971; Cardoza et al., 2006). Research into these fungi involves isolating and characterizing their phytotoxic metabolites, understanding their modes of action, and assessing their safety and effectiveness under field conditions. Such studies are essential for developing reliable, environmentally sustainable bioherbicides that can be integrated into modern agricultural practices (Whipps, 2001).

CHAPTER 09: FUTURE PROSPECTS

This study lays the groundwork for the use of phytopathogenic fungi as potential bioherbicides against *Alternanthera philoxeroides*, an aggressive invasive weed species. Although the isolation and morphological characterization of fungal pathogens provide essential preliminary data, further research is necessary to realize their application in weed management programs. A critical next step involves **evaluating the pathogenicity** of selected fungal isolates under both laboratory and natural conditions. These experiments would help determine their level of virulence, ability to infect *A. philoxeroides* consistently, and effectiveness in varying environmental settings. Additionally, **host specificity trials** are essential to ensure that these fungi do not unintentionally affect native or economically important plant species.

1. Host range studies should be performed as the promising biocontrol agent should not harm others native and economically important plant species
2. proper molecular identification of the selected fungus should be done.

The current research represents an important initial step in the identification of fungal pathogens with the potential to act as biological control agents against the invasive aquatic weed *Alternanthera philoxeroides*. While the isolation and morphological classification of these fungi is significant, further studies are needed to advance their use as reliable bioherbicides in practical field applications (Charudattan, 2001; Hoagland, 2001). Moving forward, it is essential to carry out **in vivo pathogenicity evaluations** under greenhouse and open-field conditions to determine the strength, consistency, and environmental stability of the fungal isolates. These trials will help establish whether the fungi can infect and suppress *A. philoxeroides* populations effectively, while also confirming that they do not adversely affect surrounding vegetation (Boyette et al., 2008; TeBeest et al., 1992).In addition to morphology, accurate **molecular identification methods**—such as sequencing of the ITS (Internal Transcribed Spacer) region and other genomic markers—should be utilized to confirm species-level identification and understand genetic differences among isolates (White et al., 1990; Schoch et al., 2012). This step enhances taxonomic resolution and supports reproducibility across studies. Another key focus is performing assessments on biosafety and ecological impacts. These studies would ensure that any candidate fungi pose no risk to non-target organisms, including beneficial plants, animals, and humans (Harding & Raizada, 2015). Ensuring environmental safety is crucial before advancing to field deployment.

CHAPTER 10: CONCLUSION

The fungal isolate ALI-2, identified as *Alternaria alternantherae,* has been observed to cause significant disease symptoms in *Alternanthera philoxeroides* (alligator weed) across various regions, including India. Field surveys conducted in West Bengal between 2017 and 2023 documented severe infections, with some fungal isolates inducing over 90% disease severity in the target weed. This widespread occurrence suggests that the fungus may be globally distributed, potentially spreading alongside its invasive host.

Preliminary studies have indicated that crude metabolites produced by *A. alternantherae* exhibit phytotoxic effects on *A. philoxeroides,* highlighting their potential as bioherbicidal agents. However, to advance towards commercial application, comprehensive long-term studies are essential. These investigations should focus on assessing the stability of these metabolites, their efficacy under diverse field conditions, and their overall viability as practical bioherbicides. Such research will be crucial in determining the feasibility of integrating *A. alternantherae* and its metabolites into sustainable weed management strategies (Basak et al., 2024)

CHAPTER 11: REFERENCES

Boyette, C. D., Hoagland, R. E., & Weaver, M. A. (2008). Bioherbicidal efficacy of Myrothecium verrucaria on several plant species. *Biocontrol Science and Technology*, 18(2), 103–113.

Barnett, H. L., & Hunter, B. B. (1960). *Illustrated Genera of Imperfect Fungi* (4th ed.). Burgess Publishing Company.

Boyette, C. D., Hoagland, R. E., & Horne, E. (2002). Phytotoxic activity and formulation of a crude extract of *Myrothecium verrucaria*. *Weed Technology*, **16**(2), 309–315.

Butler, E. J., & Mann, W. C. (1959). A slide-cellophane technique for observing fungal structures. *Mycologia*, **51**(4), 420–424

Charudattan, R. (2001). Biological control of weeds by means of plant pathogens: Significance for integrated weed management in modern agro-ecology. *Biocontrol*, 46(2), 229–260

Coulson, J. R. (1977). Biological control of alligator weed, *Alternanthera philoxeroides* (Martius) Griesbach, in the United States. *Agricultural Research Service Report*.

Dutta, S., Ray, S., & Ray, S. (2015). Improved methods for collection and processing of diseased aquatic weeds. *Journal of Aquatic Plant Management*, **53**, 1–7.

Dutta, S. (2021). Surface sterilization protocols for aquatic plant pathology. *Aquatic Botany Protocols*, **8**, 45–52.

Dutta, S., Das, A., & Chakraborty, P. (2023). Greenhouse protocols for twig pathogenicity tests on *Alternanthera philoxeroides*. *Phytopathologia Mediterranea*, **62**(1), 102–110.

Ellis, M. B. (1971). *Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute.

Ellis, M. B. (1976). *More Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute.

Ghosheh, H. Z., Al-Assaf, A., & Al-Fraihat, A. (2020). Invasive aquatic weeds and their impact on biodiversity. *Aquatic Botany*, 170, 103200.

Gomez, K. A., & Gomez, A. A. (1984). *Statistical Procedures for Agricultural Research* (2nd ed.). John Wiley & Sons.

Gilman, J. C. (1959). *A Manual of Soil Fungi*. Iowa State College Press.

Harding, D. P., & Raizada, M. N. (2015). Controlling weeds with fungi, bacteria and viruses: a review. *Frontiers in Plant Science*, 6, 659.

Hoagland, R. E. (2001). Microbial allelochemicals and pathogens as bioherbicidal agents. *Weed Technology*, 15(4), 835–857.

Hoagland, R. E. (2001). Formulation and bioactivity of crude fungal extracts as bioherbicides. *Biocontrol Science and Technology*, **11**(5), 517–527.

Julien, M. H., & Griffiths, M. W. (1998). *Biological control of weeds: A world catalogue of agents and their target weeds* (4th ed.). CABI Publishing.

Julien, M. H., & Broadbent, J. E. (1980). Biological control of alligator weed in Australia. *Aquatic Plant Management*, 18, 34-39.

Li, J., Wang, Q., & Zhang, Z. (2019). Fungal pathogens as biocontrol agents for managing invasive weeds: A case study on alligator weed. *Journal of Weed Science Research*, 27(2), 45-52.

Patel, S., Mehta, P., & Sharma, P. (2019). Integrated approaches for the management of Alternanthera philoxeroides in India. *Indian Journal of Weed Science*, 51(3), 238-245.

Pomella, A. W. V., Barreto, R. W., & Charudattan, R. (2007). Nimbya alternantherae: A potential biocontrol agent for alligatorweed, Alternanthera philoxeroides. *BioControl*, 52(2), 271–288.

Schoch, C. L., Seifert, K. A., Huhndorf, S., et al. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences*, 109(16), 6241–6246.

Stewart, R. M., Center, T. D., & Dray, F. A. (2000). Insects and pathogens as biological control agents of alligatorweed, Alternanthera philoxeroides. In *Proceedings of the X International Symposium on Biological Control of Weeds* (pp. 273–282).

Seefeldt, S. S., Jensen, J. E., & Fuerst, E. P. (1995). Log-logistic analysis of herbicide dose-response relationships. *Weed Technology*, **9**(2), 218–227.

Sharma, R., Singh, P., & Kaur, J. (2021). Advances in biological control of aquatic invasive weeds. *Biological Control Journal*, 58(4), 355-366.

Souza, A. N., Pereira, M. G., & Silva, F. (2017). Cold-chain management for transport of phytopathological samples. *Plant Disease*, **101**(9), 1574–1580.

TeBeest, D. O., Templeton, G. E., & Smith, R. J. (1992). Mycoherbicides: Progress in the biological control of weeds. *Plant Disease*, 76(3), 286–292.

Templeton, G. E., Smith, R. J., & Fox, W. T. (1979). Biological control of alligatorweed with Cercospora rodmanii. *Weed Science*, 27(3), 303–306.

Templeton, G. E., Wilder, G. G., & Boyette, D. E. (1979). Incidence of *Cercospora rodmanii* on alligator weed in Louisiana. *Plant Disease Reporter*, **63**(2), 122–125.

Wang, C., Chen, X., & Zhang, J. (2016). Evaluation of chemical and mechanical control methods for alligator weed management. *Weed Technology*, 30(3), 678-685

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), *PCR Protocols: A Guide to Methods and Applications* (pp. 315–322). Academic Press.

Yang, X., Zhang, Y., & Li, B. (2016). Molecular identification and bioherbicidal potential of fungal pathogens against alligatorweed (Alternanthera philoxeroides). *Biocontrol Science and Technology*, 26(5), 662–675.

Yang, Y., Li, R., & Zhang, Y. (2016). Molecular identification and metabolic profiling of *Fusarium* strains for weed biocontrol. *Journal of Agricultural and Food Chemistry*, **64**(24), 5036–5044.

Zar, J. H. (1999). *Biostatistical Analysis* (4th ed.). Prentice Hall.

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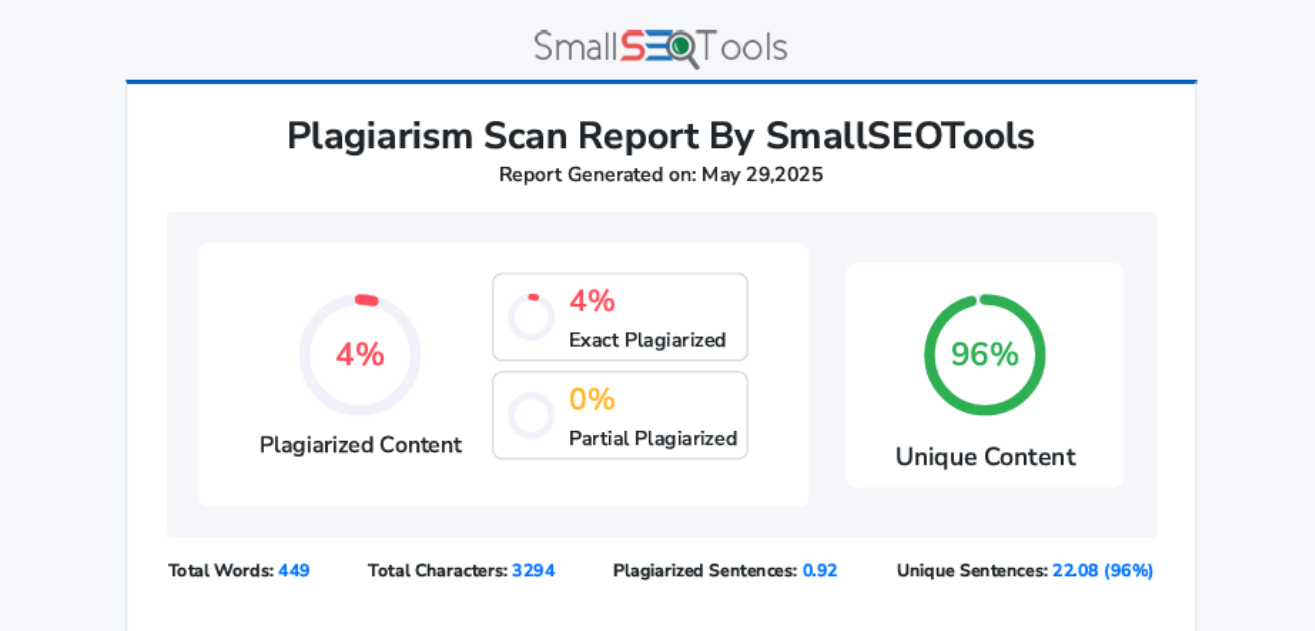

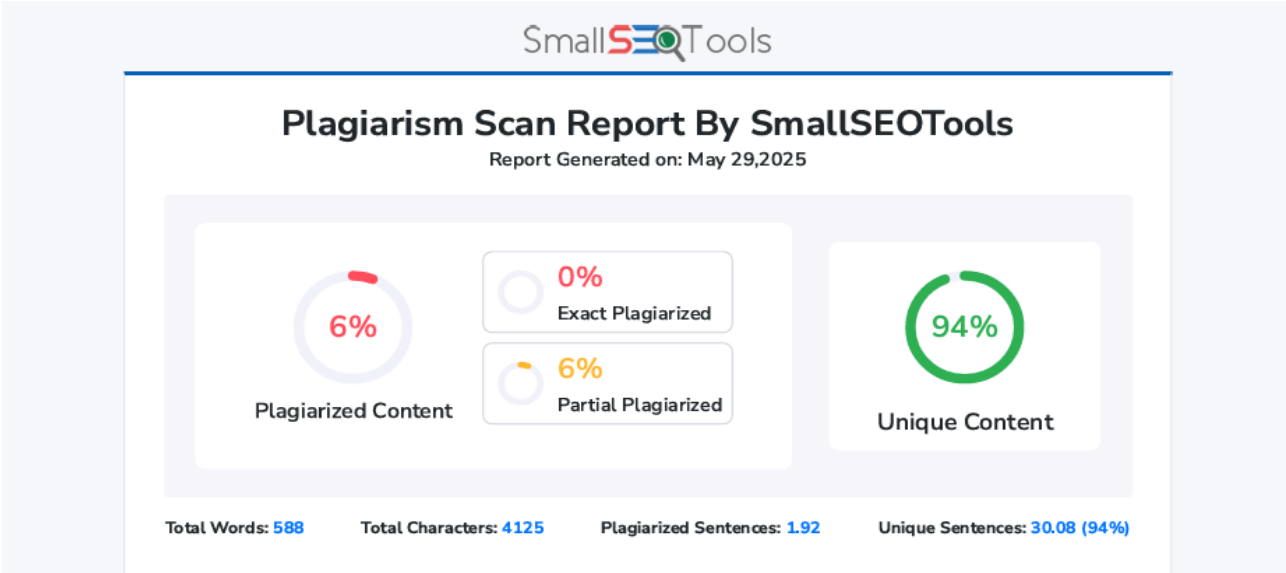

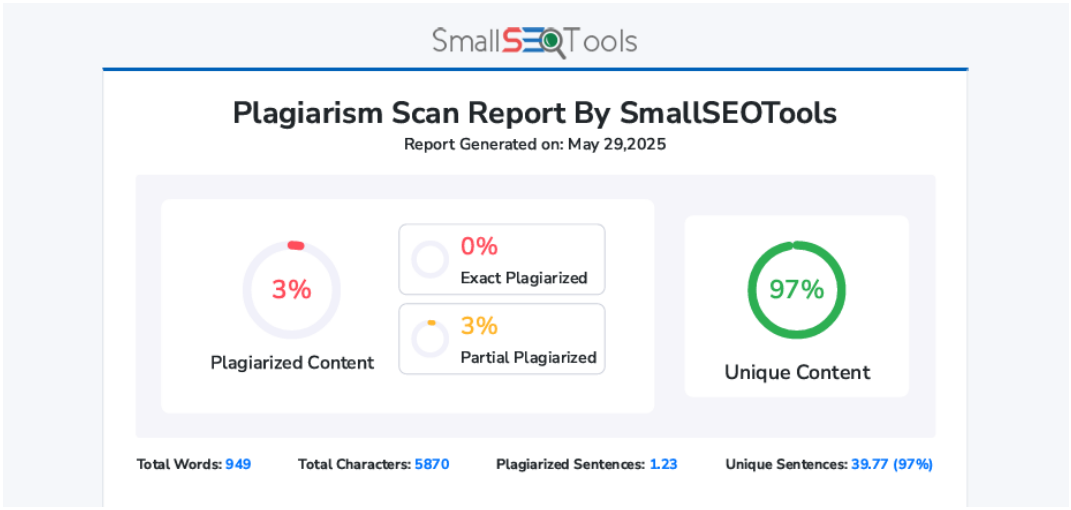
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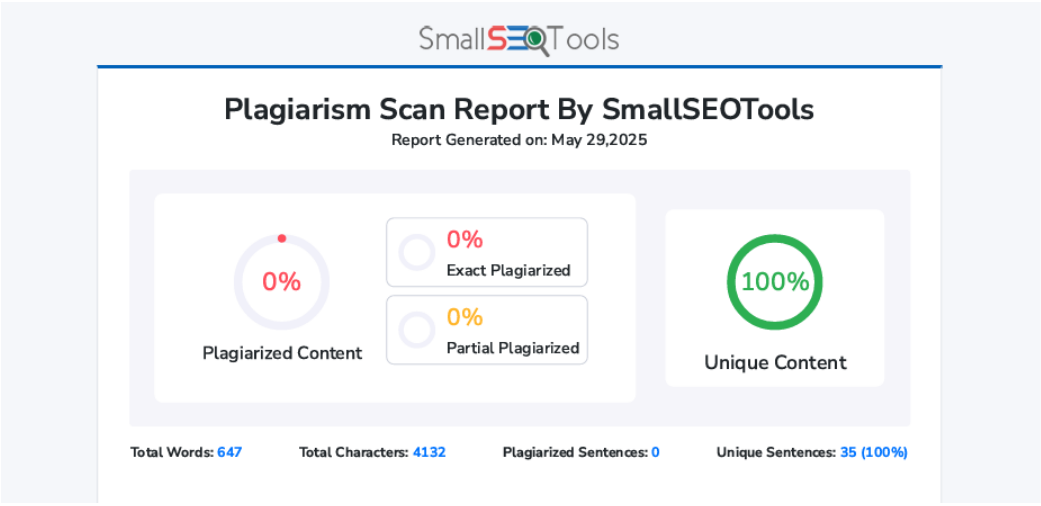
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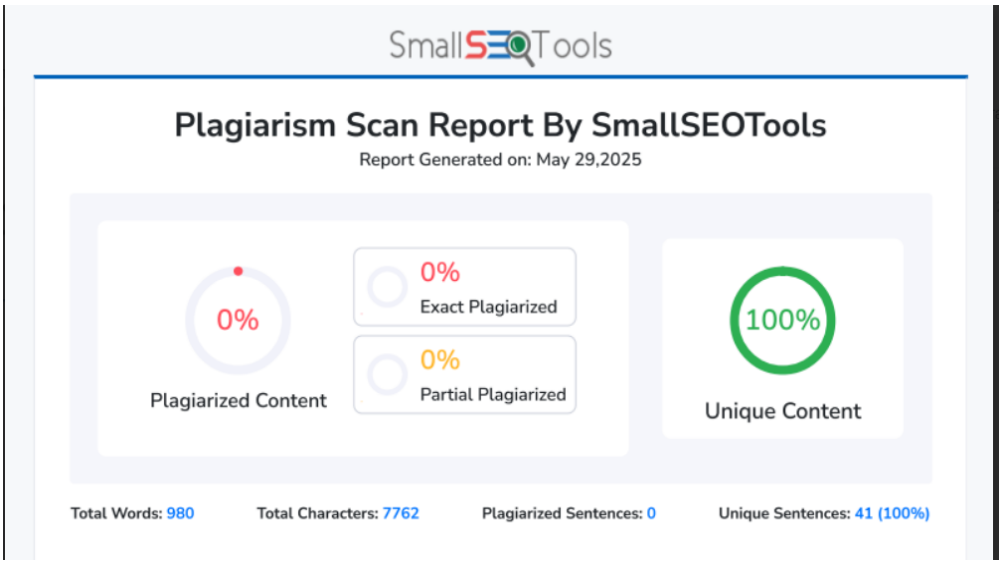
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